A Thesis for the Degree of Master of Science in Pharmacy

Isolation and Structure Determination of Bioactive Constituents from *Poncirus trifoliata* Raf.

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Abstract

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Sortase A (SrtA), a gram-positive transpeptidase, is an important target in reducing bacterial infection. The extract from the fruits of trifoliate orange, *Poncirus trifoliata* Rafinesque, showed significant Srt A inhibition.

Poncirus trifoliata Raf. is a member of the family Rutaceae and it has been widely used in Korean traditional medicine for the treatment of diverse pathogenic symptoms.

Bioactivity-guided chromatographic separation afforded the isolation of in total twelve compounds, eight coumarins and four flavanone glycosides.

The structures of four novel coumarins were determined by a combination of spectroscopic analyses such as NMR and LC-MS.

The absolute configurations of the new coumarins and known

coumarins, previously unassigned, were determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method.

Key words: herbal medicine, *Poncirus trifoliata* Rafinesque, coumarins, flavanone glycosides, sortase A inhibition

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Introduction

Sortase A (SrtA), a gram-positive transpeptidase, plays a key role in the pathogenesis of gram-positive bacteria by adhesion to the host cell via the covalent anchoring of virulence-associated proteins to cell wall peptioglycans.[1] Accordingly, inhibitors of SrtA might be promising candidates for the treatment and prevention of gram-positive bacterial infection.[2]

In the preparatory study on the SrtA inhibition test from Korean traditional medicine, approximately two-hundreds of plant extracts were tested. Among them, the extract from the fruits of trifoliate orange, *Poncirus trifoliata* Rafinesque, showed significant Srt A inhibition.

Poncirus trifoliata Rafinesque, a member of the family Rutaceae, is a deciduous or semi-deciduous shrub native to China and Korea.[3] *Poncirus trifoliata* Raf. are widely used in oriental traditional medicine as a remedy for digestive ulcers, gastritis, emesis, inflammation, allergy, and dysentery.[4] Also, diverse bio-activity was reported.[5-10]

The organic extracts from *Poncirus trifoliata* Raf. were separated by employing solvent-partitioning. Bioactivity-guided chromatographic separation afforded the isolation of in total twelve compounds, eight coumarins and four flavanone glycosides. Among the isolated compounds,

four comarins were defined to be new natural products. The structures of four novel coumarins were determined by a combination of spectroscopic analyses.

The absolute configurations of the new coumarins (**1-4**) and known coumarins (**5** and **6**), previously unassigned, were determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method.[11]

Experimental section

1. General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter and using a 1 cm cell. CD data were obtained on a JASCO J-715 spectropolarimeter in MEOD solutions. IR spectra were obtained on a JASCO FT/IR-300E spectrophotometer. UV spectra were recorded on a Hitachi U-3010 spectrophotometer. NMR spectra were recorded in $CDCl_3$, DMSO, and CD_3OD solutions, on a Bruker AMX-500 and 125 MHz, respectively. Mass spectra were provided by the Korea Basic Science Institute, Daegu Branch, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

2. Plant Material

Poncirus trifoliata Raf. were purchased from the Kyungdong-Market, Seoul, Korea, in April, 2016. A voucher specimen is on deposit at the Natural Products Research Institute, College of Pharmacy, Seoul National University.

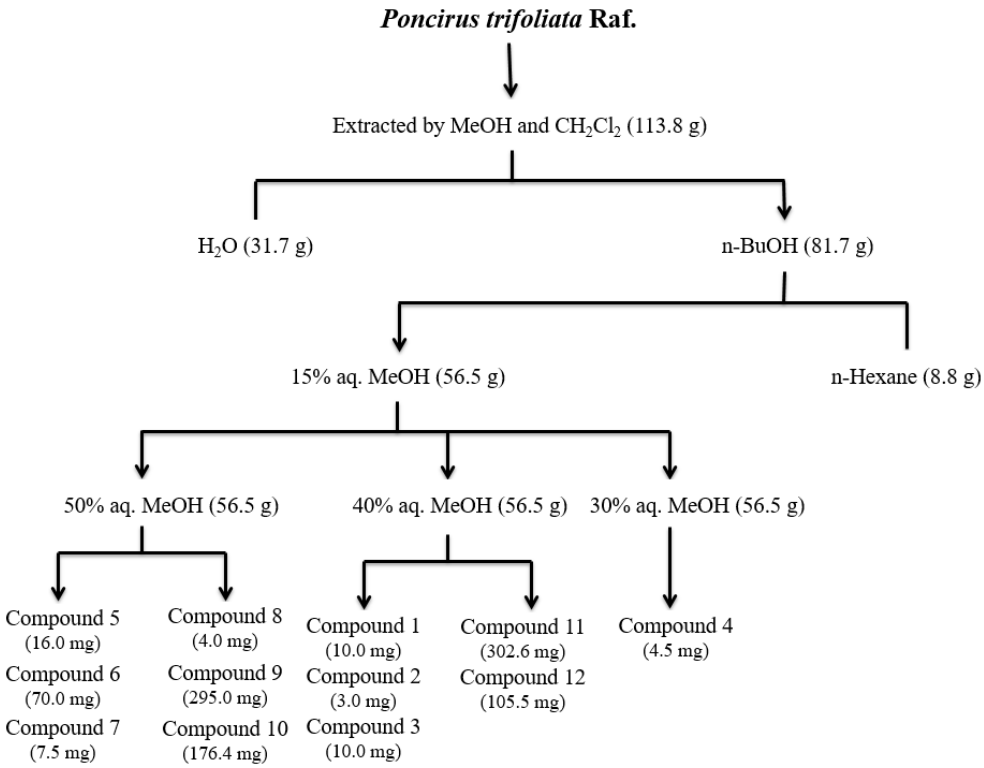
3. Extraction and Isolation

Poncirus trifolata Raf. was extracted with Methanol (10 L X 3) and CH_2Cl_2 (10L X 3). The combined crude extract (113.8 g) were partitioned between H_2O (31.7 g) and n-buthanol (81.7 g). The latter fraction was repartitioned between n-hexane (8.8 g) and 15% aqueous methanol (56.5 g). 15% aqueous methanol layer from solvent partitioning was subjected to C_{18} reversed-phase vacuum flash chromatography sequential moxtures of H_2O and Methanol (elution order: 50%, 40%, 30%, 20%, 10% aqueous methanol, 100% methanol), 100% acetone, and finally 100% ethyl acetate.

Based on the result of ^1H NMR and bioactivity test, the fractions eluted with 50% aqueous methanol (5.6 g). 40% aqueous methanol (1.0 g), 30% aqueous methanol (1.1 g) were chosen for separation. The fraction eluted with 50% aqueous methanol was separated by semi-preparative reversed-phase HPLC (YMC-ODS column, 10mm X 250mm; 70% aqueous methanol) to afford 16.0, 70.0, 7.5, 4.0, 295.0, and 176.4 mg of Compounds **5, 6, 7, 8, 9,** and **10,** respectively.

The fraction eluted with 40% aqueous methanol was separated by semi-preparative reversed-phase HPLC (YMC-ODS column, 10mm X 250mm; 53% aqueous methanol) to afford 10.0, 3.0, 10.0, 302.6, and 105.5 mg of Compounds **1, 2, 3, 11,** and **12,** respectively.

The fraction eluted with 30% aqueous methanol was separated by semi-preparative reversed-phase HPLC (YMC-ODS column, 10mm X 250mm; 43% aqueous methanol) to afford 4.5 mg of Compounds **4.**



Scheme 1. Isolation of compounds from *Poncirus trifoliata* Raf.

4. Preparation of the (*S*)- and (*R*)-α-Methoxy-α-trifluoromethylphenylacetic chloride (MTPA-Cl) Esterification of Compound 1-6

Reactions were performed as follows. To duplicate solution of **1** (0.9 mg, 2 μmol each) and DMAP (0.5 mg each) in 0.8 mL of anhydrous pyridine were added (*R*)- or (*S*)-MTPA chloride (8 mg, 0.03 mmol each). The mixture was allowed to stand under N_2 at room temperature for 2 h. After reaction, samples were dried under vacuum and separated by HPLC (YMS-ODS column, 4.6mm X 250 mm; 25% aqueous methanol) to afford the pure (*S*)- and (*R*)-MTPA esters derivatives of **1**. The (*S*)- and (*R*)-MTPA esters of **2-6** were also prepared by similar method.

^{1}H NMR (Chloroform-*d*, 600 MHz) data of **1S**: δ_H 7.5579 (1H, d, *J* = 9.7 Hz, H-4), 7.54-7.17 (10H, m, MTPA-Ar), 7.2897 (1H, d, *J* = 8.2 Hz, H-5), 6.7043 (1H, d, *J* = 8.7 Hz, H-6), 6.1848 (1H, d, *J* = 9.4 Hz, H-3), 5.5459 (1H, dd, *J* = 2.6, 7.7 Hz, H-2′), 5.4605 (1H, dd, *J* = 2.3, 11.5 Hz, H-2″), 4.1992 (2H, m, H-1′), 3.5223 (3H, s, MTPA-OMe), 3.2726 (3H, s, MTPA-OMe), 3.2278 (1H, dd, *J* = 11.9, 14.0 Hz, H-1″a), 3.1831 (3H, s, OMe), 2.9657 (1H, dd, *J* = 2.5, 14.3 Hz, H-1″b), 1.3393 (3H, s, H-4″), 1.2582 (3H, s, H-5″), 1.0995 (3H, s, H-4′), 1.0803 (3H, s, H-5′); LRESIMS *m*/*z* 835.1 $[M + Na]^+$ (calcd for $C_{40}H_{42}F_6O_{11}$, 812.3).

^{1}H NMR (Chloroform-*d*, 600 MHz) data of **1R:** δ_H 7.5976 (1H, d, *J* = 9.7 Hz, H-4), 7.55-7.15 (10H, m, MTPA-Ar), 7.2912 (1H, d, *J* = 8.2 Hz, H-5),

6.7522 (1H, d, *J* = 8.7 Hz, H-6), 6.2389 (1H, d, *J* = 9.4 Hz, H-3), 5.5914 (1H, dd, *J* = 2.6, 7.7 Hz, H-2′), 5.4443 (1H, dd, *J* = 2.3, 11.5 Hz, H-2′′), 4.1945 (2H, m, H-1′), 3.5054 (3H, s, MTPA-OMe), 3.4186 (3H, s, MTPA-OMe), 3.3904 (1H, dd, *J* = 11.9, 14.0 Hz, H-1′′a), 3.2123(3H, s, OMe), 3.1119 (1H, dd, *J* = 2.5, 14.3 Hz, H-1′′b), 1.3086 (3H, s, H-4′′), 1.2179 (3H, s, H-5′′), 1.1948 (3H, s, H-4′), 1.1883 (3H, s, H-5′); LRESIMS *m*/*z* 835.1 $[M + Na]^+$ (calcd for $C_{40}H_{42}F_6O_{11}$, 812.3).

^{1}H NMR (Methanol-d_4, 600 MHz) data of **2S:** δ_H 7.8684 (1H, d, *J* = 9.1 Hz, H-4), 7.54-7.17 (10H, m, MTPA-Ar), 7.5188 (1H, d, *J* = 8.7 Hz, H-5), 6.9172 (1H, d, *J* = 8.7 Hz, H-6), 6.2450 (1H, d, *J* = 9.2 Hz, H-3), 5.8427 (1H, dd, *J* = 2.8, 11.4 Hz, H-2′), 5.4390 (1H, dd, *J* = 2.3, 7.9 Hz, H-2′′), 4.2344 (2H, m, H-1′′), 3.5424 (3H, s, MTPA-OMe), 3.2945 (3H, s, OMe), 3.2353 (3H, s, MTPA-OMe), 3.2142 (1H, dd, *J* = 11.3, 14.0 Hz, H-1′a), 2.7834 (1H, dd, *J* = 3.1, 14.1 Hz, H-1′b), 1.3268 (3H, s, H-4′′), 1.3268 (3H, s, H-5′′), 1.1349 (3H, s, H-5′), 1.1402 (3H, s, H-4′); LRESIMS *m*/*z* 857.2 $[M - H + HCO_2H]^-$ (calcd for $C_{40}H_{42}F_6O_{11}$, 812.3).

^{1}H NMR (Methanol-d_4, 600 MHz) data of **2R:** δ_H 7.8971 (1H, d, *J* = 9.1 Hz, H-4), 7.56-7.04 (10H, m, MTPA-Ar), 7.5533 (1H, d, *J* = 8.7 Hz, H-5), 6.9830 (1H, d, *J* = 8.7 Hz, H-6), 6.2785 (1H, d, *J* = 9.2 Hz, H-3), 5.8257 (1H, dd, *J* = 2.8, 11.4 Hz, H-2′), 5.5402 (1H, dd, *J* = 2.3, 7.9 Hz, H-2′′), 4.2781 (2H, m, H-1′′), 3.5713 (3H, s, MTPA-OMe), 3.4219 (1H, dd, *J* = 11.3, 14.0 Hz, H-1′a), 3.3685 (3H, s, MTPA-OMe), 3.2677 (3H, s, OMe),

2.9286 (1H, dd, *J* = 3.1, 14.1 Hz, H-1′b), 1.3076 (3H, s, H-4′′), 1.2993 (3H, s, H-5′′), 1.2770 (3H, s, H-5′), 1.2686 (3H, s, H-4′); LRESIMS *m*/*z* 857.2 $[M - H + HCO_2H]^-$ (calcd for $C_{40}H_{42}F_6O_{11}$, 812.3).

^{1}H NMR (Methanol-d_4, 600 MHz) data of **3S:** δ_H 7.9072 (1H, d, *J* = 9.6 Hz, H-4), 7.5747 (1H, d, *J* = 8.7 Hz, H-5), 7.55-7.27 (5H, m, MTPA-Ar), 7.0787 (1H, d, *J* = 8.7 Hz, H-6), 6.2597 (1H, d, *J* = 9.6 Hz, H-3), 5.5363 (1H, dd, *J* = 1.8, 8.8 Hz, H-2′), 4.4349 (1H, dd, *J* = 1.8, 10.5 Hz, H-1′a), 4.3364 (1H, dd, *J* = 8.8, 10.8 Hz, H-1′b), 3.9171 (1H, d, *J* = 17.8 Hz, H-1′′a), 3.8415 (1H, d, *J* = 18.0 Hz, H-1′′b), 3.5264 (3H, s, MTPA-OMe), 3.2195 (3H, s, OMe), 2.8185 (1H, m, H-3′′), 1.1914 (3H, d, *J* = 6.9 Hz, H-4′′), 1.1668 (3H, d, *J* = 7.1 Hz, H-5′′), 1.1607 (3H, s, H-4′), 1.1361 (3H, s, H-5′); LRESIMS *m*/*z* 601.2 $[M + Na]^+$ (calcd for $C_{30}H_{33}F_3O_8$, 578.2).

^{1}H NMR (Methanol-d_4, 600 MHz) data of **3R:** δ_H 7.8988 (1H, d, *J* = 9.6 Hz, H-4), 7.54-7.27 (5H, m, MTPA-Ar), 7.5321 (1H, d, *J* = 8.7 Hz, H-5), 6.9934 (1H, d, *J* = 8.7 Hz, H-6), 6.2601 (1H, d, *J* = 9.6 Hz, H-3), 5.5556 (1H, dd, *J* = 1.8, 8.8 Hz, H-2′), 4.3332 (1H, dd, *J* = 1.8, 10.5 Hz, H-1′a), 4.2047 (1H, dd, *J* = 8.8, 10.8 Hz, H-1′b), 4.004 (1H, d, *J* = 17.8 Hz, H-1′′a), 3.9599 (1H, d, *J* = 18.0 Hz, H-1′′b), 3.5406 (3H, s, MTPA-OMe), 3.2664 (3H, s, OMe), 2.8688 (1H, m, H-3′′), 1.2732 (3H, s, H-4′), 1.2432 (3H, s, H-5′), 1.2220 (3H, d, *J* = 6.9 Hz, H-4′′), 1.1829 (3H, d, *J* = 7.1 Hz, H-5′′); LRESIMS *m*/*z* 601.2 $[M + Na]^+$ (calcd for $C_{30}H_{33}F_3O_8$, 578.2).

^{1}H NMR (DMSO-d_6, 500 MHz) data of **4S:** δ_H 8.0136 (1H, d, *J* = 9.6 Hz, H-4), 7.6402 (1H, d, *J* = 8.6 Hz, H-5), 7.48-7.40 (5H, m, MTPA-Ar), 7.1648 (1H, d, *J* = 8.7 Hz, H-6), 6.3064 (1H, d, *J* = 9.5 Hz, H-3), 5.7644 (1H, dd, *J* = 2.7, 7.5 Hz, H-2′), 5.0210 (1H, s, H-4′a), 4.9536 (1H, s, H-4′b), 4.4049 (1H, dd, *J* = 7.7, 11.1 Hz, H-1′a), 4.3158 (1H, dd, *J* = 2.8, 11.1 Hz, H-1′b), 3.8741 (2H, s, H-1′′), 3.4610 (3H, s, MTPA-OMe), 2.7374 (1H, m, H-3′′), 1.6729 (3H, s, H-5′), 1.0778 (3H, d, *J* = 6.9 Hz, H-4′′), 1.0606 (3H, d, *J* = 6.9 Hz, H-5′′); LRESIMS *m*/*z* 569.2 $[M + Na]^+$ (calcd for $C_{29}H_{29}F_3O_7$, 546.2).

^{1}H NMR (DMSO-d_6, 500 MHz) data of **4R:** δ_H 8.0010 (1H, d, *J* = 9.6 Hz, H-4), 7.6075 (1H, d, *J* = 8.6 Hz, H-5), 7.46-7.39 (5H, m, MTPA-Ar), 7.1111 (1H, d, *J* = 8.7 Hz, H-6), 6.2998 (1H, d, *J* = 9.5 Hz, H-3), 5.7632 (1H, dd, *J* = 2.7, 7.5 Hz, H-2′), 5.1695 (1H, s, H-4′a), 5.1243 (1H, s, H-4′b), 4.3548 (1H, dd, *J* = 7.7, 11.1 Hz, H-1′a), 4.2828 (1H, dd, *J* = 2.8, 11.1 Hz, H-1′b), 3.8213 (2H, s, H-1′′), 3.4312 (3H, s, MTPA-OMe), 2.7309 (1H, m, H-3′′), 1.8053 (3H, s, H-5′), 1.0829 (3H, d, *J* = 6.9 Hz, H-4′′), 1.0516 (3H, d, *J* = 6.9 Hz, H-5′′); LRESIMS *m*/*z* 569.2 $[M + Na]^+$ (calcd for $C_{29}H_{29}F_3O_7$, 546.2).

^{1}H NMR (Methanol-d_4, 500 MHz) data of **5S:** δ_H 7.8802 (1H, d, *J* = 9.6 Hz, H-4), 7.55-7.11 (10H, m, MTPA-Ar), 7.5222 (1H, d, *J* = 8.7 Hz, H-5), 6.9033 (1H, d, *J* = 8.6 Hz, H-6), 6.2437 (1H, d, *J* = 9.5 Hz, H-3), 5.6641 (1H, dd, *J* = 2.5, 11.7 Hz, H-2′), 5.4254 (1H, dd, *J* = 1.9, 8.5 Hz, H-2′′),

4.2004 (2H, m, H-1″), 3.5436 (3H, s, MTPA-OMe), 3.3494 (3H, s, MTPA-OMe), 3.2435 (1H, dd, *J* = 1.7, 3.3 Hz, H-1′a), 2.8924 (1H, dd, *J* = 2.2, 13.9 Hz, H-1′b), 1.3513 (3H, s, H-4″), 1.3242 (3H, s, H-5″), 1.1295 (3H, s, H-4′), 1.1244 (3H, s, H-5′); LRESIMS *m*/*z* 843.1 $[M - H + HCO_2H]^-$ (calcd for $C_{39}H_{40}F_6O_{11}$, 798.3).

^{1}H NMR (Methanol-d_4, 500 MHz) data of **5R:** δ_H 7.9166 (1H, d, *J* = 9.6 Hz, H-4), 7.57-7.06 (10H, m, MTPA-Ar), 7.5578 (1H, d, *J* = 8.7 Hz, H-5), 6.9856 (1H, d, *J* = 8.6 Hz, H-6), 6.2909 (1H, d, *J* = 9.5 Hz, H-3), 5.6799 (1H, dd, *J* = 2.5, 11.7 Hz, H-2′), 5.5543 (1H, dd, *J* = 1.9, 8.5 Hz, H-2″), 4.2593 (2H, m, H-1″), 3.5736 (3H, s, MTPA-OMe), 3.4080 (3H, s, MTPA-OMe), 3.4464 (1H, dd, *J* = 1.7, 3.3 Hz, H-1′a), 3.0482 (1H, dd, *J* = 2.2, 13.9 Hz, H-1′b), 1.3299 (3H, s, H-4″), 1.2972 (3H, s, H-5″), 1.2729 (3H, s, H-4′), 1.2729 (3H, s, H-5′); LRESIMS *m*/*z* 843.1 $[M - H + HCO_2H]^-$ (calcd for $C_{39}H_{40}F_6O_{11}$, 798.3).

^{1}H NMR (Methanol-d_4, 600 MHz) data of **6S:** δ_H 7.9102 (1H, d, *J* = 9.6 Hz, H-4), 7.5797 (1H, d, *J* = 8.7 Hz, H-5), 7.56-7.26 (5H, m, MTPA-Ar), 7.0812 (1H, d, *J* = 8.7 Hz, H-6), 6.2606 (1H, d, *J* = 9.6 Hz, H-3), 5.4438 (1H, dd, *J* = 1.6, 9.1 Hz, H-2′), 4.5132 (1H, dd, *J* = 1.4, 10.9 Hz, H-1′a), 4.3540 (1H, dd, *J* = 9.1, 10.5 Hz, H-1′b), 3.9109 (1H, d, *J* = 17.8 Hz, H-1″a), 3.8498 (1H, d, *J* = 18.0 Hz, H-1″b), 3.5338 (3H, s, MTPA-OMe), 2.8174 (1H, m, H-3″), 1.1896 (3H, d, *J* = 6.9 Hz, H-4″), 1.1665 (3H, d, *J* = 6.9 Hz, H-5″), 1.1590 (3H, s, H-4′), 1.1580 (3H, s, H-5′); LRESIMS *m*/*z* 565.2 $[M + H]^+$ (calcd for

$C_{29}H_{31}F_3O_8$, 564.2).

^{1}H NMR (Methanol-d_4, 600 MHz) data of **6R:** δ_H 7.8974 (1H, d, J = 9.6 Hz, H-4), 7.5339 (1H, d, J = 8.7 Hz, H-5), 7.53-7.26 (5H, m, MTPA-Ar), 6.9936 (1H, d, J = 8.7 Hz, H-6), 6.2592 (1H, d, J = 9.6 Hz, H-3), 5.4209 (1H, dd, J = 1.6, 9.1 Hz, H-2′), 4.3943 (1H, dd, J = 1.4, 10.9 Hz, H-1′a), 4.2122 (1H, dd, J = 9.1, 10.5 Hz, H-1′b), 3.9874 (1H, d, J = 17.8 Hz, H-1′′a), 3.9469 (1H, d, J = 18.0 Hz, H-1′′b), 3.5409 (3H, s, MTPA-OMe), 2.8574 (1H, m, H-3′′), 1.2761 (3H, s, H-4′), 1.2612 (3H, s, H-5′), 1.2139 (3H, d, J = 6.9 Hz, H-4′′), 1.1736 (3H, d, J = 6.9 Hz, H-5′′); LRESIMS m/z 565.2 $[M + H]^+$ (calcd for $C_{29}H_{31}F_3O_8$, 564.2).

Results

1. Compound 1

Compound **1** was obtained as a yellow amorphous powder. The ^{1}H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.60 (1H, d, *J* = 9.6 Hz), 7.30 (1H, d, *J* = 8.7 Hz), 6.85 (1H, d, *J* = 8.7 Hz), and 6.22 (1H, d, *J* = 9.2 Hz), methoxy signal at δ_H 3.24 (3H, s).

Absolute configuration of C-2′ and C-2′′ were determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method (Figure 31).

Based upon the result of combined spectroscopic analyses, the structure of compound **1** was determined to be a new coumarin possessing methoxy group.

2. Compound 2

Compound **2** was obtained as a yellow amorphous powder. The ^{1}H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.88 (1H, d, *J* = 9.2 Hz), 7.48 (1H, d, *J* = 8.7 Hz), 7.05 (1H, d, *J* = 8.7 Hz), and 6.24 (1H, d, *J* = 9.6 Hz), methoxy signal at δ_H 3.24 (3H, s).

Absolute configuration of C-2′ and C-2′′ were determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method

(figure 31).

Based upon the result of combined spectroscopic analyses, the structure of compound **2** was determined to be a new coumarin possessing methoxy group.

3. Compound 3

Compound **3** was obtained as a yellow amorphous powder. The ^{1}H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.86 (1H, d, J = 9.5 Hz), 7.51 (1H, d, J = 8.6 Hz), 7.04 (1H, d, J = 8.6 Hz), and 6.21 (1H, d, J = 9.5 Hz), methoxy signal at δ_H 3.26 (3H, s). ^{13}C NMR spectrum indicated a ketone signal at δ_C 214.0.

Absolute configuration of C-2′ was determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method (figure 31).

Based upon the result of combined spectroscopic analyses, the structure of compound **3** was determined to be a new coumarin possessing methoxy group.

4. Compound 4

Compound **4** was obtained as a yellow amorphous powder. The ^{1}H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 8.00 (1H, d, J = 9.5 Hz), 7.61 (1H, d, J = 8.6 Hz), 7.10 (1H, d, J = 8.6 Hz), and 6.28 (1H, d, J = 9.6 Hz), methylene signals at δ_H 4.87 (1H, s) and 5.02

(1H, s). ^{13}C NMR spectrum indicated a ketone signal at δ_C 210.0.

Absolute configuration of C-2′ was determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method (figure 31).

Based upon the result of combined spectroscopic analyses, the structure of compound **4** was determined to be a new coumarin possessing exo-methylene.

5. Compound 5

Compound **5** was obtained as a yellow amorphous powder. The ^1H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.90 (1H, d, *J* = 9.5 Hz), 7.49 (1H, d, *J* = 8.5 Hz), 7.05 (1H, d, *J* = 8.6 Hz), and 6.25 (1H, d, *J* = 9.4 Hz).

Absolute configuration of C-2′ and C-2′′ were determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method (figure 31).

Based upon the result of combined spectroscopic analyses, the structure of compound **5** was identified to be ponciol.

6. Compound 6

Compound **6** was obtained as a yellow amorphous powder. The ^1H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.88 (1H, d, *J* = 9.4 Hz), 7.54 (1H, d, *J* = 8.7 Hz), 7.06 (1H, d, *J* = 8.6 Hz),

and 6.23 (1H, d, J = 9.4 Hz). ^{13}C NMR spectrum indicated a ketone signal at δ_C 214.1.

Absolute configuration of C-2′ was determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method (figure 31).

Based upon the result of combined spectroscopic analyses, the structure of compound **6** was identified to be triphasiol.[12]

7. Compound 7

Compound **7** was obtained as a colorless crystal. The ^{1}H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.85 (1H, d, J = 9.4 Hz), 7.45 (1H, d, J = 8.5 Hz), 6.79 (1H, dd, J = 2.3, 8.6 Hz), and 6.18 (1H, d, J = 9.4 Hz).

Based upon the result of combined spectroscopic analyses, the structure of compound **7** was identified to be umbelliferone.[13,14]

8. Compound 8

Compound **8** was obtained as white neddles. The ^{1}H NMR spectrum indicated a coumarin structure with aromatic signals at δ_H 7.59 (1H, d, J = 16.0 Hz), 7.45 (1H, d, J = 8.6 Hz), 6.80 (1H, d, J = 8.6 Hz), and 6.29 (1H, d, J = 15.9 Hz).

Based upon the result of combined spectroscopic analyses, the structure of compound **8** was identified to be umbelliferone.[15]

9. Compound 9

Compound **9** was obtained as a yellow powder. The ^{1}H NMR spectrum indicated a flavanone structure with aromatic signals at δ_H 7.30 (2H, d, *J* = 8.3 Hz), 6.82 (2H, d, *J* = 8.3 Hz), 6.17 (1H, d, *J* = 2.3 Hz), and 6.15 (1H, d, *J* = 2.3 Hz).

Based upon the result of combined spectroscopic analyses, the structure of compound **9** was identified to be naringin.[16,17]

10. Compound 10

Compound **10** was obtained as a yellow powder. The ^{1}H NMR spectrum indicated a flavanone structure with aromatic signals at δ_H 7.32 (2H, d, *J* = 8.3 Hz), 6.82 (2H, d, *J* = 8.3 Hz), 6.20 (1H, d, *J* = 2.3 Hz), and 6.17 (1H, d, *J* = 2.3 Hz).

Based upon the result of combined spectroscopic analyses, the structure of compound **10** was identified to be narirutin.[18,19]

11. Compound 11

Compound **11** was obtained as a white powder. The ^{1}H NMR spectrum indicated a flavanone structure with aromatic signals at δ_H 7.45 (2H, d, *J* = 8.7 Hz), 6.98 (2H, d, *J* = 8.7 Hz), 6.14 (1H, d, *J* = 2.1 Hz), and 6.10 (1H, d, *J* = 2.1 Hz).

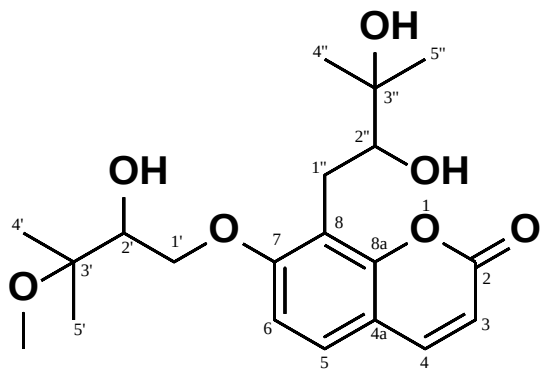
Based upon the result of combined spectroscopic analyses, the structure of

compound **11** was identified to be poncirin.[4,20]

12. Compound 12

Compound **12** was obtained as a white powder. The ^{1}H NMR spectrum indicated a flavanone structure with aromatic signals at δ_H 7.47 (2H, d, *J* = 8.7 Hz), 6.98 (2H, d, *J* = 8.7 Hz), 6.14 (1H, d, *J* = 2.3 Hz), and 6.13 (1H, d, *J* = 2.3 Hz).

Based upon the result of combined spectroscopic analyses, the structure of compound **11** was identified to be neoponcirin.[21,22,23]

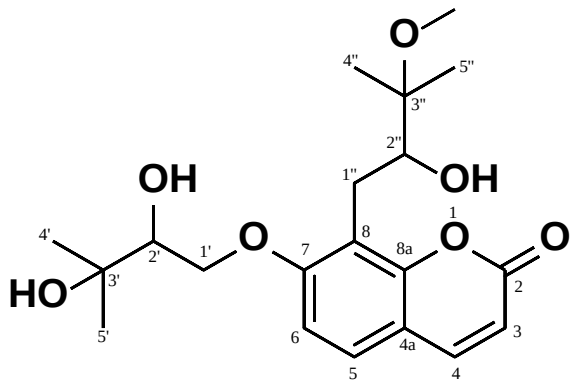


1

Table 1. ^{13}C and ^{1}H NMR assignment for compound **1** in Chloroform-*d*

Position	δ_C	δ_H
2	161.3, C	
3	113.4, CH	6.22, d (9.2)
4	144.0, CH	7.60, d (9.6)
4a	113.3, C	
5	127.0, CH	7.30, d (8.7)
6	108.7, CH	6.85, d (8.7)
7	159.9, C	
8	116.7, C	
8a	153.6, C	
1′	69.6, CH_2	4.08, dd (7.8, 10.1)
		4.35, dd (2.7, 10.2)
2′	74.9, CH	3.81, dd (2.8, 7.4)
3′	76.4, C	
4′	21.7, CH_3	1.23, s
5′	20.6, CH_3	1.22, s
1″	25.6, CH_2	2.99, dd (8.7, 13.8)
		3.12, dd (2.4, 13.7)

2″	78.8, CH	3.64, dd (2.3, 10.1)
3″	73.6, C	
4″	25.7, CH_3	1.34, s
5″	24.7, CH_3	1.29, s
OMe	49.6, CH_3	3.24, s

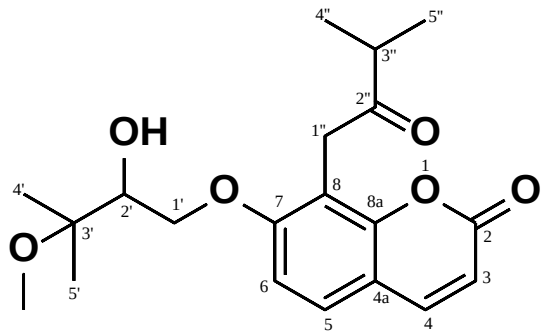


2

Table 2. ^{13}C and ^{1}H NMR assignment for compound **2** in Methanol-d_4

Position	δ_C	δ_H
2	161.7, C	
3	113.3, CH	6.24, d (9.6)
4	146.4, CH	7.88, d (9.2)
4a	114.6, C	
5	128.5, CH	7.48, d (8.7)
6	110.2, CH	7.05, d (8.7)
7	161.9, C	
8	118.0, C	
8a	154.9, C	
1′	71.4, CH_2	4.07, dd (7.8, 10.1)
		4.42, dd (2.8, 10.1)
2′	77.3, CH	3.76, dd (2.8, 7.8)
3′	72.8, C	
4′	27.2, CH_3	1.28, s
5′	25.1, CH_3	1.25, s
1′′	26.2, CH_2	3.09, m
2′′	77.4, CH	3.79, dd (3.9, 8.9)

3″	79.3, C	
4″	22.0, CH_3	1.29, s
5″	20.5, CH_3	1.29, s
OMe	50.0, CH_3	3.32, s

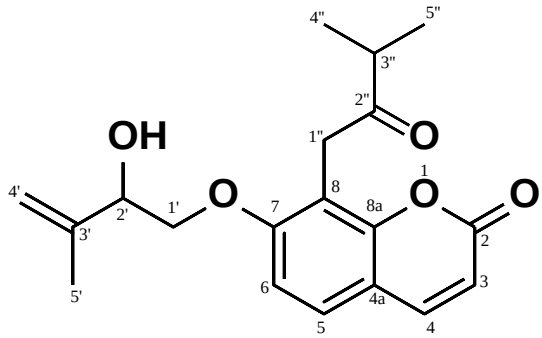


3

Table 3. ^{13}C and ^{1}H NMR assignment for compound **3** in Methanol-d_4

Position	δ_C	δ_H
2	163.1, C	
3	113.3, CH	6.21, d (9.5)
4	146.2, CH	7.86, d (9.5)
4a	114.4, C	
5	129.3, CH	7.51, d (8.6)
6	110.1, CH	7.04, d (8.6)
7	161.6, C	
8	113.1, C	
8a	154.5, C	
1′	71.9, CH_2	4.01, dd (8.2, 10.1)
		4.33, dd (2.4, 10.2)
2′	76.5, CH	3.78, dd (2.3, 8.1)
3′	77.8, C	
4′	22.4, CH_3	1.24, s
5′	20.4, CH_3	1.21, s
1′′	36.0, CH_2	4.09, d (1.4)
2′′	214.0, C	
3′′	42.0, CH	2.90, m

4″	18.9, CH_3	1.18, d (1.4)
5″	19.0, CH_3	1.20, d (1.2)
OMe	49.9, CH_3	3.26, s

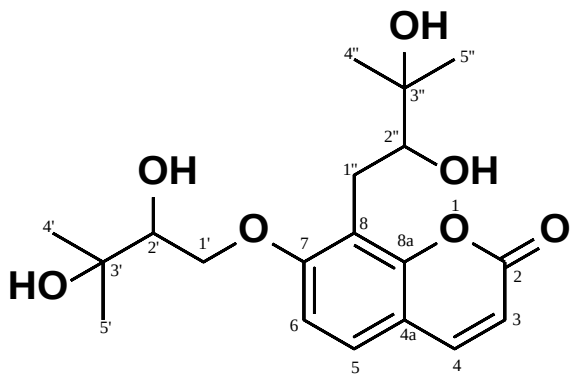


4

Table 4. ^{13}C and ^{1}H NMR assignment for compound **4** in DMSO-d_6

Position	δ_C	δ_H
2	160.0, C	
3	111.9, CH	6.28, d (9.6)
4	144.7, CH	8.00, d (9.5)
4a	112.5, C	
5	128.0, CH	7.61, d (8.6)
6	108.9, CH	7.10, d (8.6)
7	159.4, C	
8	112.3, C	
8a	152.8, C	
1′	71.5, CH_2	3.99, dd (2.7, 6.4)
		4.04, dd (4.7, 9.8)
2′	72.2, CH	4.23, t (5.4)
3′	145.0, C	
4′	111.5, CH_2	4.87, s
		5.02, s
5′	18.0, CH_3	1.70, s
1′′	34.4, CH_2	3.95, d (4.7)
2′′	210.0, C	

3″	39.6, CH	2.82, m
4″	18.2, CH_3	1.11, d (2.1)
5″	18.1, CH_3	1.09, d (2.1)

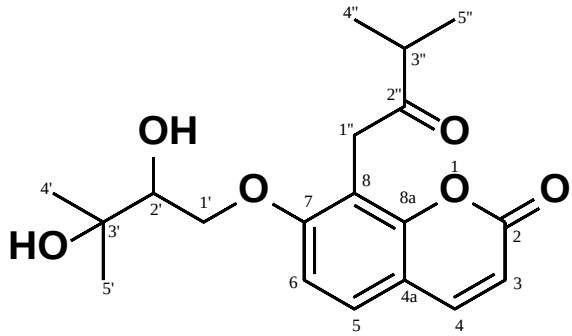


5

Table 5. ^{13}C and ^{1}H NMR assignment for compound **5** in Methanol-d_4

Position	δ_C	δ_H
2	163.9, C	
3	113.2, CH	6.25, d (9.4)
4	146.5, CH	7.90, d (9.5)
4a	114.6, C	
5	128.5, CH	7.49, d (8.5)
6	110.2, CH	7.05, d (8.6)
7	162.0, C	
8	118.0, C	
8a	154.9, C	
1′	71.5, CH_2	4.07, dd (8.1, 10.1)
		4.43, dd (2.8, 10.2)
2′	77.4, CH	3.77, dd (2.8, 8.0)
3′	72.9, C	
4′	27.3, CH_3	1.29, s
5′	25.0, CH_3	1.25, s
1′′	26.5, CH_2	3.10, m
2′′	79.2, CH	3.69, dd (3.5, 9.2)

3″	74.4, C	
4″	26.3, CH_3	1.31, s
5″	24.8, CH_3	1.30, s

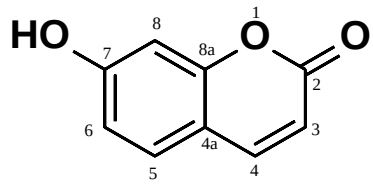


6

Table 6. ^{13}C and ^{1}H NMR assignment for compound **6** in Methanol-d_4

Position	δ_C	δ_H
2	163.1, C	
3	113.4, CH	6.23, d (9.4)
4	146.2, CH	7.88, d (9.4)
4a	114.5, C	
5	129.4, CH	7.54, d (8.7)
6	110.1, CH	7.06, d (8.6)
7	161.7, C	
8	113.1, C	
8a	154.6, C	
1′	71.9, CH_2	4.03, dd (8.2, 10.2)
		4.37, dd (2.4, 10.0)
2′	77.8, CH	3.71, dd (2.5, 8.1)
3′	72.8, C	
4′	27.0, CH_3	1.26, s
5′	25.0, CH_3	1.22, s
1″	36.0, CH_2	4.12, d (6.4)
2″	214.1, C	
3″	42.0, CH	2.91, m

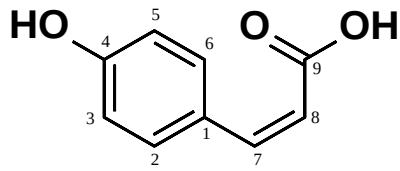
4″	18.9, CH_3	1.20, d (2.3)
5″	19.0, CH_3	1.21, d (2.3)



7

Table 7. ^{13}C and ^{1}H NMR assignment for compound **7** in Methanol-d_4

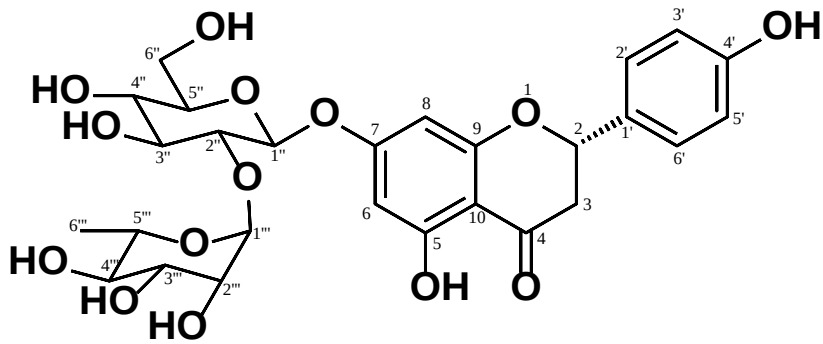
Position	δ_C	δ_H
2	163.8, C	
3	114.6, CH	6.18, d (9.4)
4	146.2, CH	7.85, d (9.4)
4a	113.3, C	
5	130.8, CH	7.45, d (8.5)
6	112.5, CH	6.79, dd (2.3, 8.5)
7	163.3, C	
8	103.5, C	6.71, d (2.3)
8a	157.4, C	



8

Table 8. ^{13}C and ^{1}H NMR assignment for compound **8** in Methanol-d_4

Position	δ_C	δ_H
1	127.5, C	
2	131.2, CH	7.45, d (8.6)
3	116.9, CH	6.80, d (8.6)
4	161.3, C	
5	116.9, CH	6.80, d (8.6)
6	131.2, CH	7.45, d (8.6)
7	146.5, CH	7.59, d (16.0)
8	116.1, CH	6.29, d (15.9)
9	171.4, C	



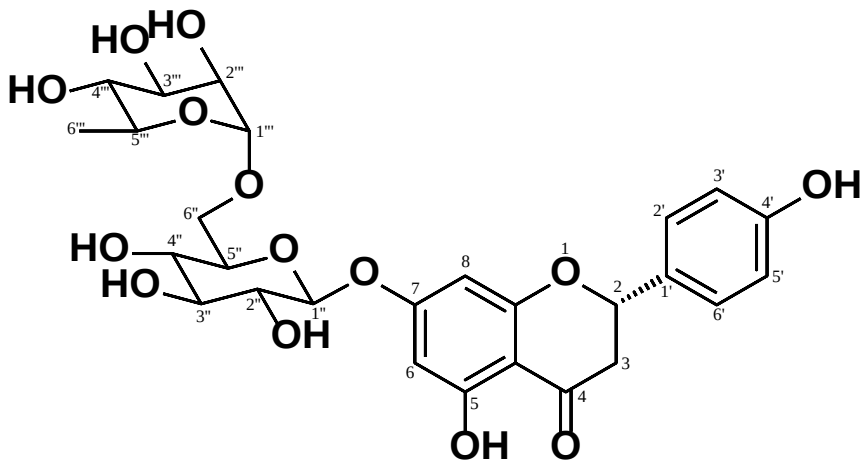
9

Table 9. ^{13}C and ^{1}H NMR assignment for the aglycone moiety of compound **9** in Methanol-d_4

Position	δ_C	δ_H
2	80.8, CH	5.33, dd (2.3, 12.8)
3	44.2, CH_2	2.73, dd (2.3, 17.4)
		3.14, dd (12.8, 17.4)
4	198.6, C	
5	165.1, C	
6	98.0, CH	6.15, d (2.3)
7	166.7, C	
8	96.9, CH	6.17, d (2.3)
9	164.7, C	
10	105.0, C	
1′	130.9, C	
2′	129.2, CH	7.30, d (8.3)
3′	116.5, CH	6.82, d (8.3)
4′	159.2, C	
5′	116.5, CH	6.82, d (8.3)
6′	129.2, CH	7.30, d (8.7)

Table 10. ^{13}C NMR assignment for the sugar moieties of compound **9** in Methanol-d_4

Position	δ_C
Glc 1″	99.5
2″	79.1
3″	79.0
4″	71.3
5″	78.2
6″	62.4
Rha 1‴	102.6
2‴	72.3
3‴	72.3
4‴	74.0
5‴	70.1
6‴	18.3



10

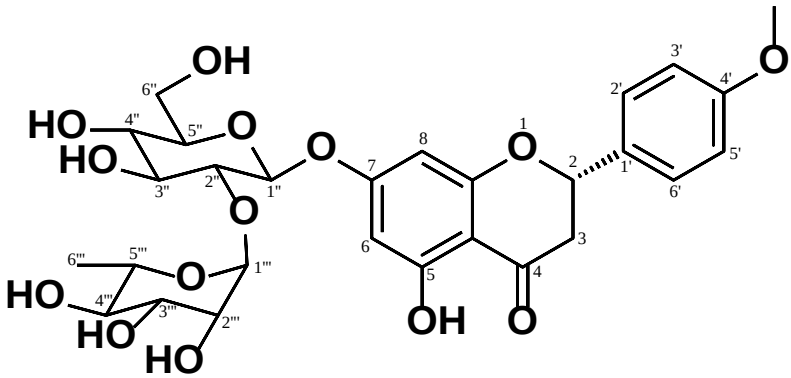
Table 11. ^{13}C and ^{1}H NMR assignment for the aglycone moiety of compound **10** in Methanol-d_4

Position	δ_C	δ_H
2	80.7, CH	5.38, dd (2.3, 12.8)
3	44.2, CH_2	2.74, dd (2.3, 17.4)
		3.14, dd (12.8, 17.4)
4	198.6, C	
5	165.1, C	
6	98.1, CH	6.17, d (2.3)
7	166.9, C	
8	97.3, CH	6.20, d (2.3)
9	159.2, C	
10	105.1, C	
1′	131.0, C	
2′	129.3, CH	7.32, d (8.3)
3′	116.5, CH	6.82, d (8.3)

4′	164.5, C	
5′	116.5, CH	6.82, d (8.3)
6′	129.3, CH	7.32, d (8.3)

Table 12. ^{13}C NMR assignment for the sugar moieties of compound **10** in Methanol-d_4

Position	δ_C
Glc 1″	101.3
2″	74.8
3″	78.0
4″	71.4
5″	77.2
6″	67.5
Rha 1‴	102.2
2‴	72.2
3‴	72.5
4‴	74.2
5‴	69.9
6‴	18.0



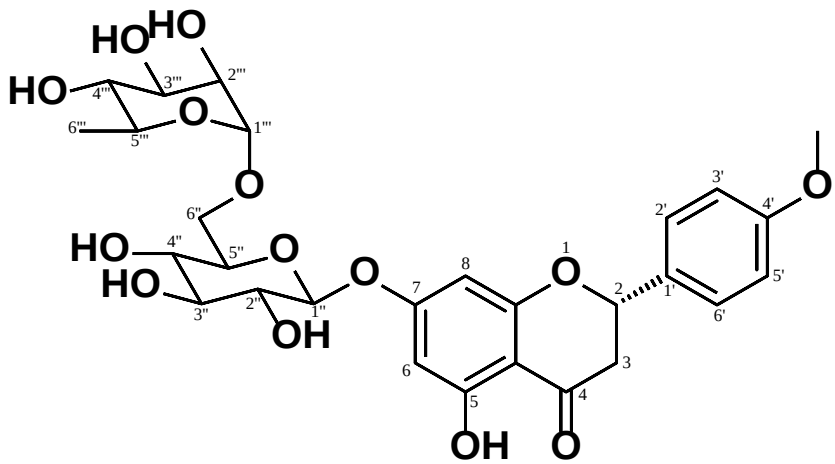
11

Table 13. ^{13}C and ^{1}H NMR assignment for the aglycone moiety of compound **11** in DMSO-d_6

Position	δ_C	δ_H
2	78.4, CH	5.59, dd (2.7, 12.7)
3	42.1, CH_2	2.78, dd (2.7, 17.2)
		3.19, m
4	197.1, C	
5	163.0, C	
6	96.4, CH	6.10, d (2.1)
7	164.9, C	
8	95.2, CH	6.14, d (2.1)
9	162.7, C	
10	103.4, C	
1′	130.4, C	
2′	128.4, CH	7.45, d (8.7)
3′	114.0, CH	6.98, d (8.7)
4′	159.6, C	
5′	114.0, CH	6.98, d (8.7)
6′	128.4, CH	7.45, d (8.7)
OMe	55.2, CH_3	3.77, s

Table 14. ^{13}C NMR assignment for the sugar moieties of compound **11** in DMSO-d_6

Position	δ_C
Glc 1″	100.4
2″	77.2
3″	76.1
4″	69.6
5″	76.9
6″	60.5
Rha 1‴	97.5
2‴	70.5
3‴	70.4
4‴	71.9
5‴	68.3
6‴	18.1



12

Table 15. ^{13}C and ^{1}H NMR assignment for the aglycone moiety of compound **12** in DMSO-d_6

Position	δ_C	δ_H
2	78.3, CH	5.58, dd (2.9, 12.6)
3	41.9, CH_2	2.78, dd (2.8, 17.2)
		3.13, m
4	197.0, C	
5	163.0, C	
6	96.4, CH	6.13, d (2.3)
7	165.1, C	
8	95.4, CH	6.14, d (2.3)
9	162.5, C	
10	103.3, C	
1′	103.3, C	
2′	128.4, CH	7.47, d (8.7)
3′	113.9, CH	6.98, d (8.7)

4′	159.5, C	
5′	113.9, CH	6.98, d (8.7)
6′	128.4, CH	7.47, d (8.7)
OMe	55.2, CH_3	3.77, s

Table 16. ^{13}C NMR assignment for the sugar moieties of compound **12** in DMSO-d_6

Position	δ_C
Glc 1″	99.4
2″	72.9
3″	76.2
4″	69.6
5″	75.5
6″	66.0
Rha 1‴	100.6
2‴	70.3
3‴	70.7
4‴	72.1
5‴	68.3
6‴	17.9

Discussion

The previous phytochemical investigation of *Poncirus trifoliata* Rafinesque has afforded diverse secondary metabolites such as flavonoids, coumarins, terpenoids. Despite diverse bioactivity of *Poncirus trifoliata* Raf. was reported, however, sortase A which is an enzyme that plays a key role in cell wall protein anchoring and virulence in *Staphylococcus aureus* inhibitory activity was not researched.

In this study, bioactivity-guided chromatographic separation afforded the isolation of in total twelve compounds, eight coumarins and four flavanone glycosides. Among the isolated compounds, the structures of four new coumarins were determined by a combination of spectroscopic analyses.

In addition to determination of the plannar structure, the absolute configuration of the new coumarins and known coumarins, previously unassigned, were determined by an application of the 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) method. Detailed sortase A inhibitory activity of these compounds will be evaluated.

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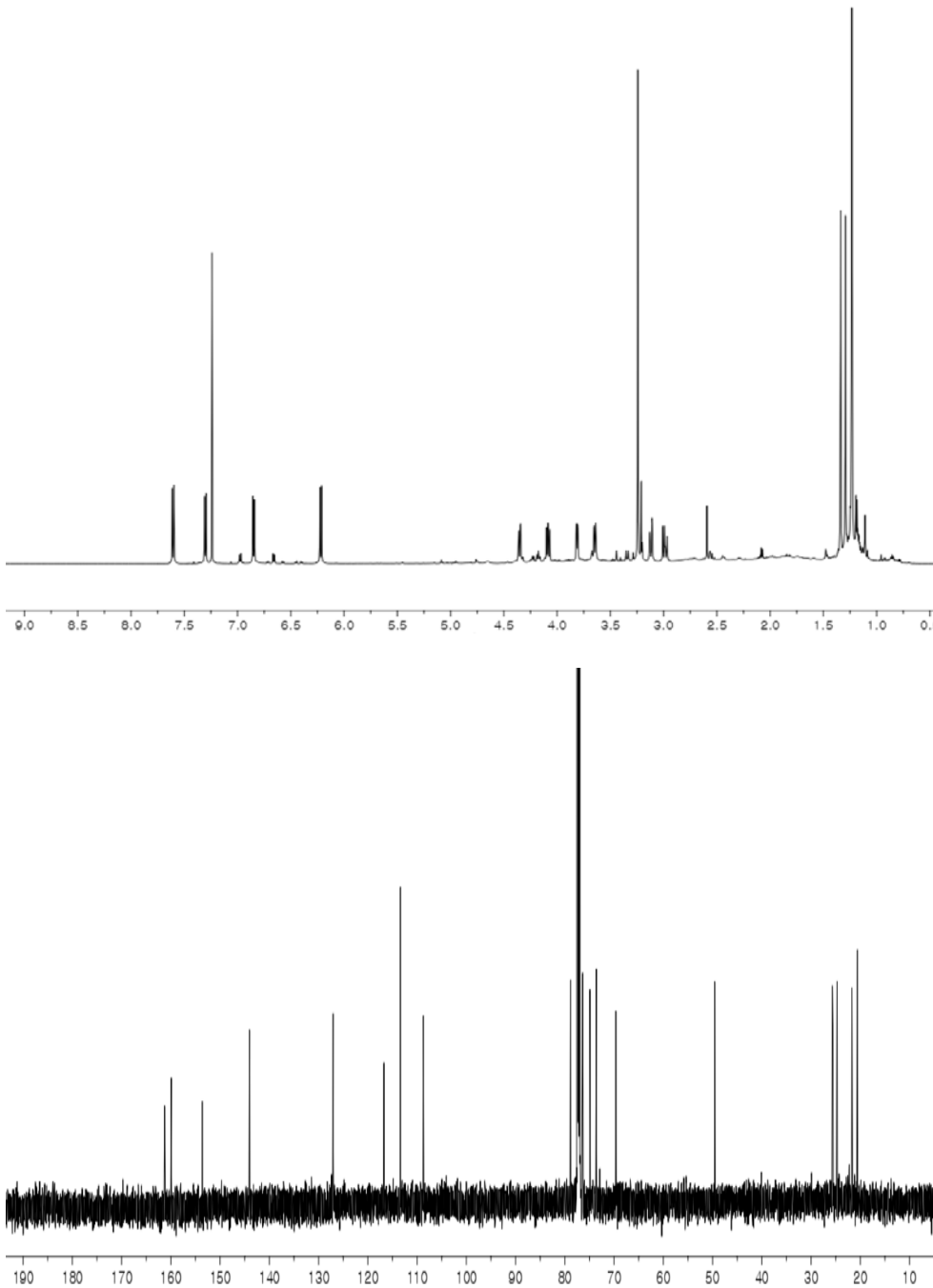

Figure 1. ^{1}H and ^{13}C NMR spectrum of compound **1**

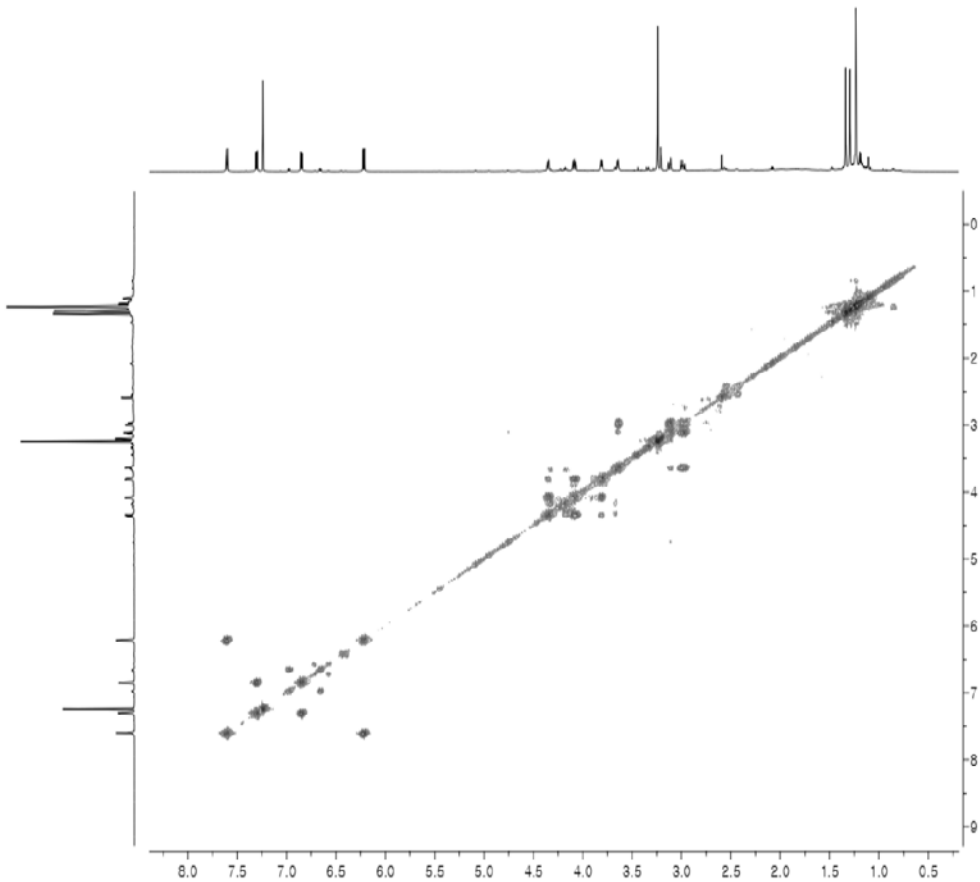

Figure 2. COSY spectrum of compound **1**

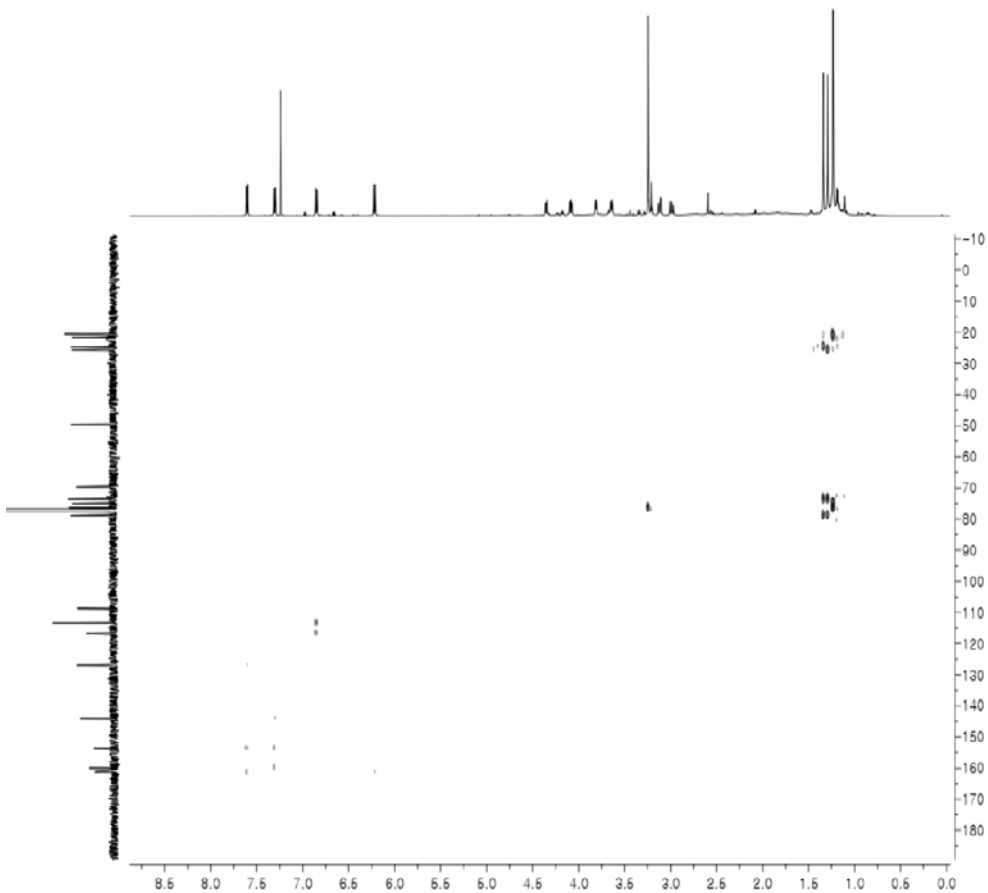

Figure 3. HMBC spectrum of compound **1**

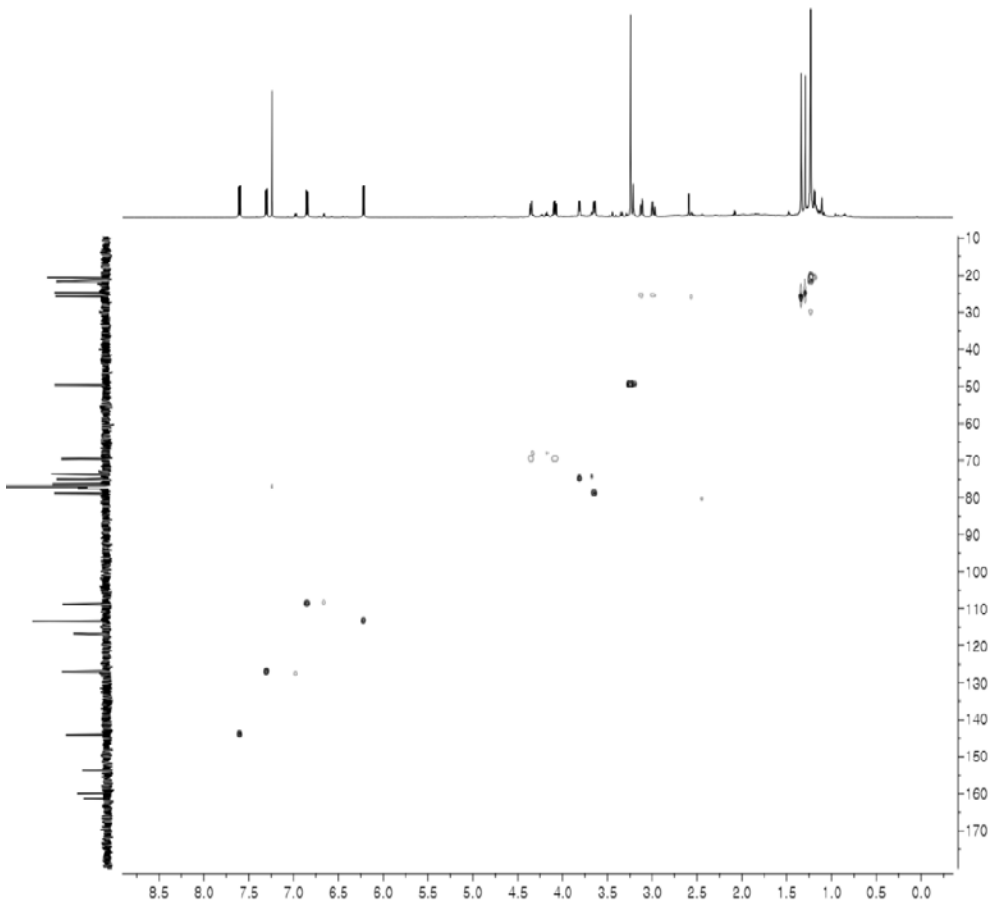

Figure 4. HSQC spectrum of compound **1**

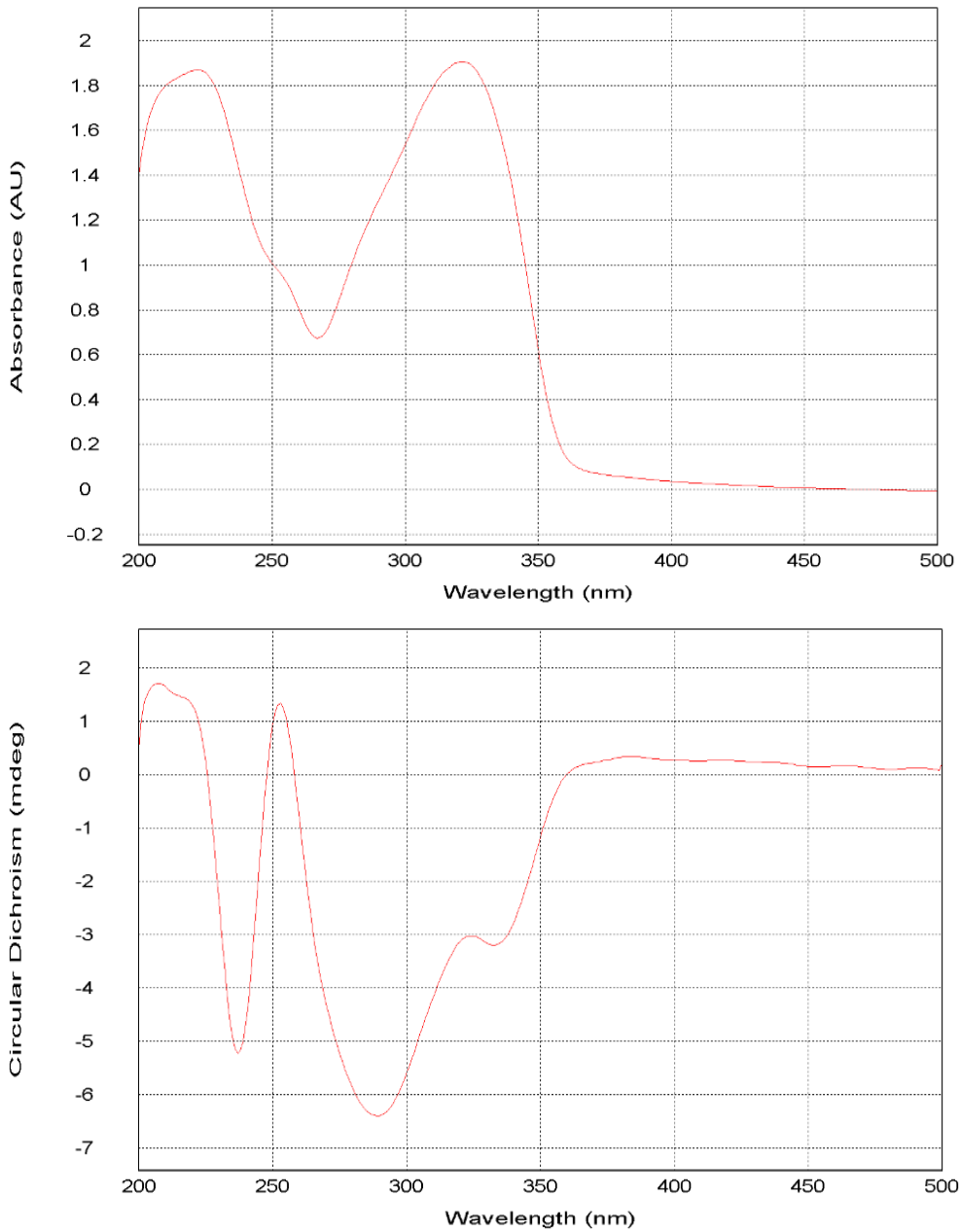

Figure 5. UV and CD spectrum of compound **1**

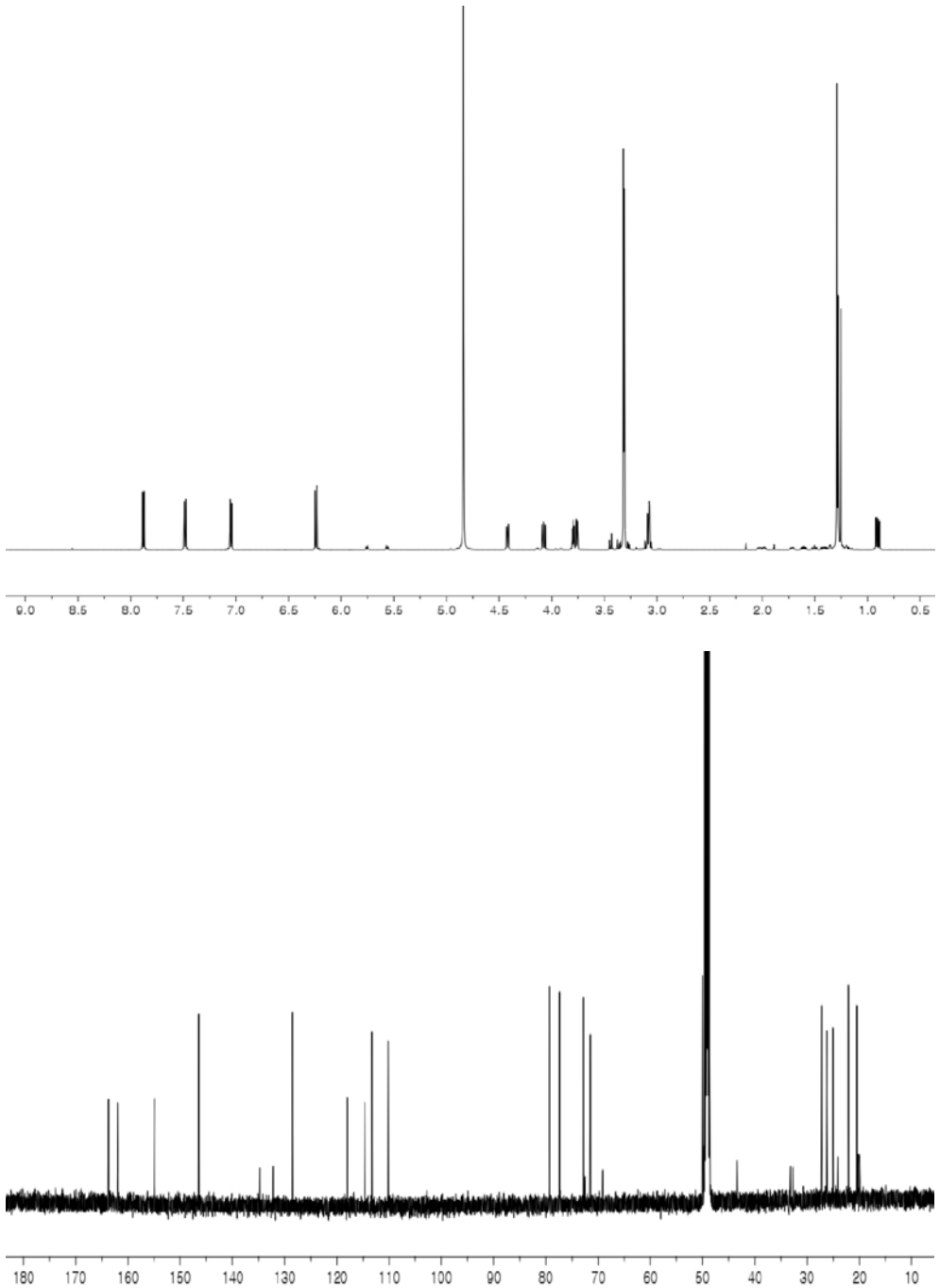

Figure 6. ^{1}H and ^{13}C NMR spectrum of compound **2**

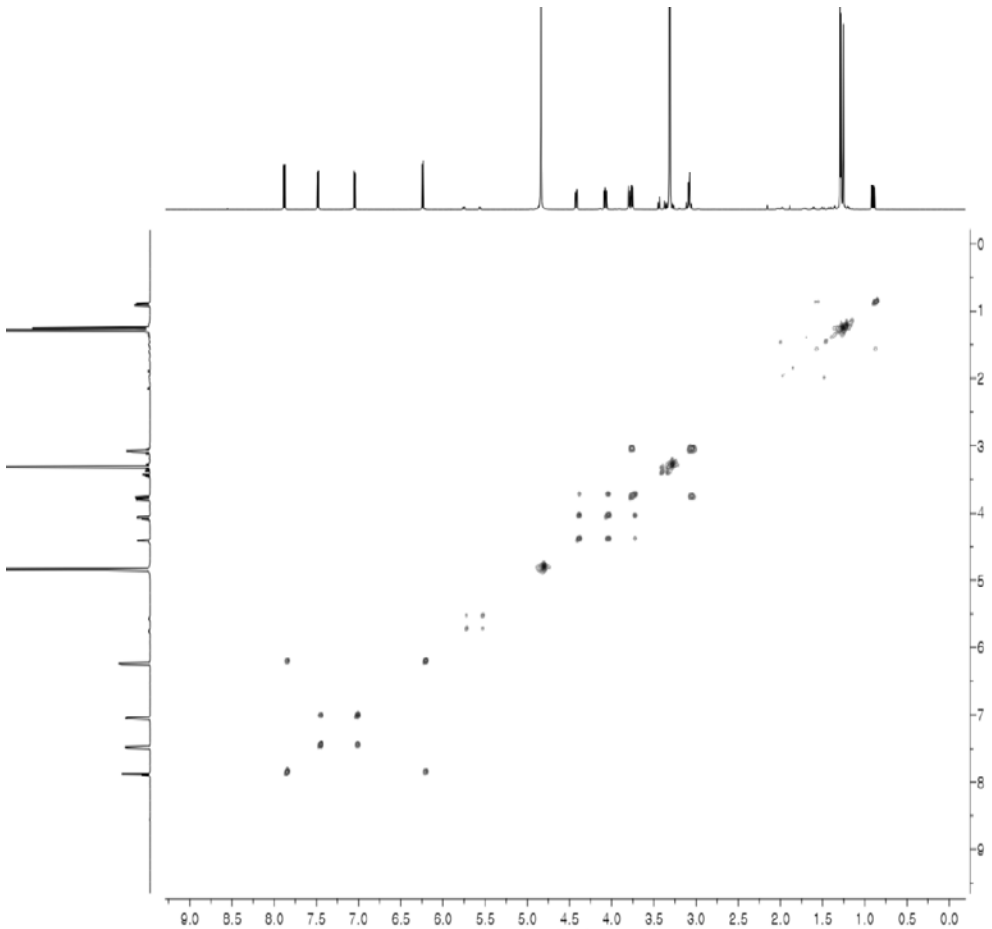

Figure 7. COSY spectrum of compound **2**

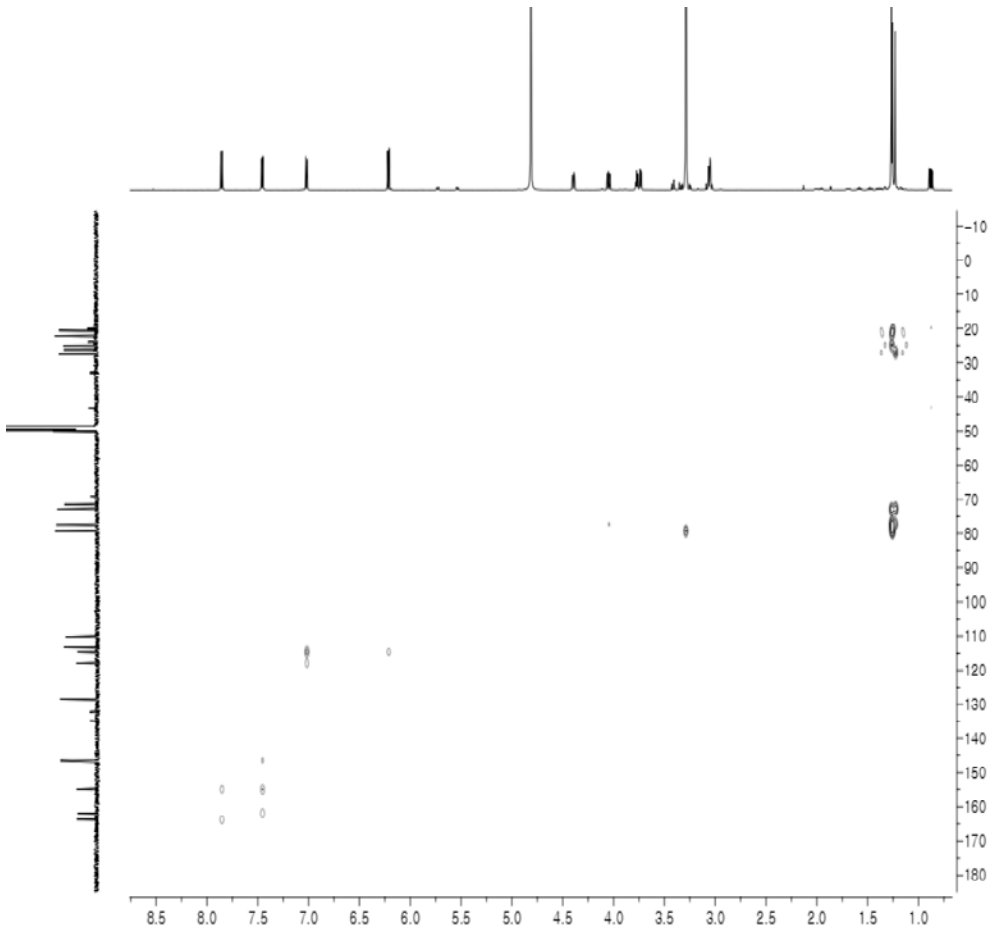

Figure 8. HMBC spectrum of compound **2**

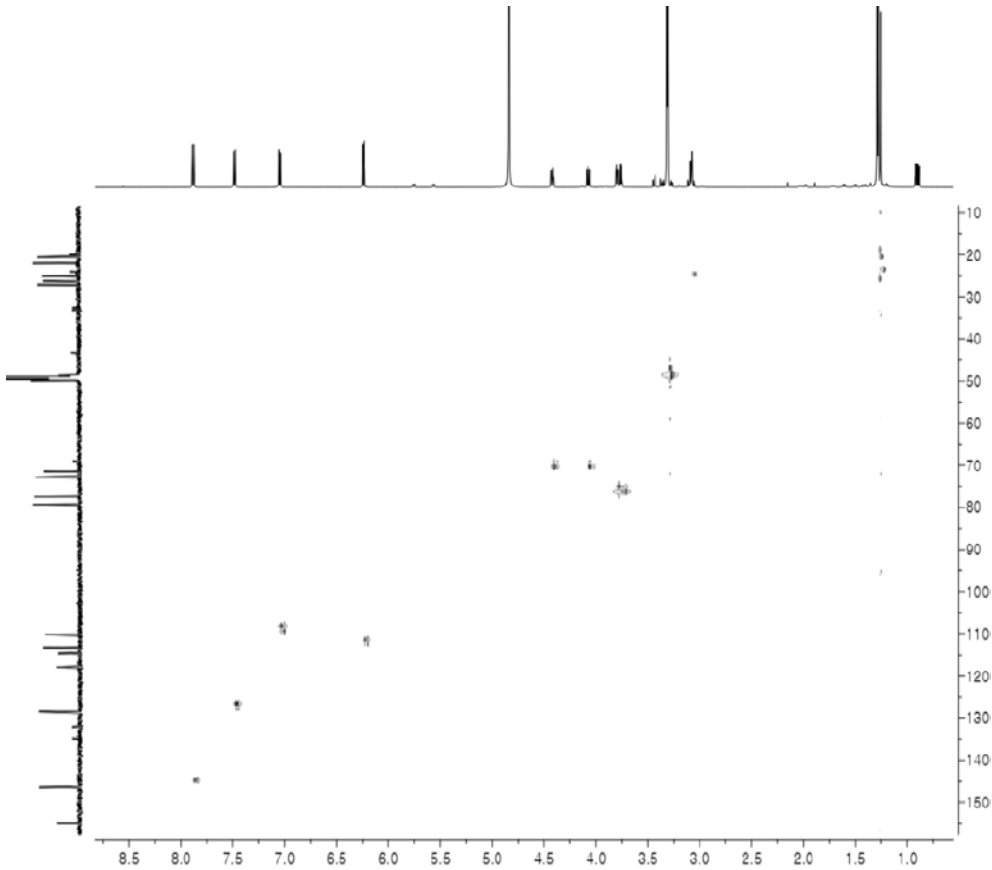

Figure 9. HSQC spectrum of compound **2**

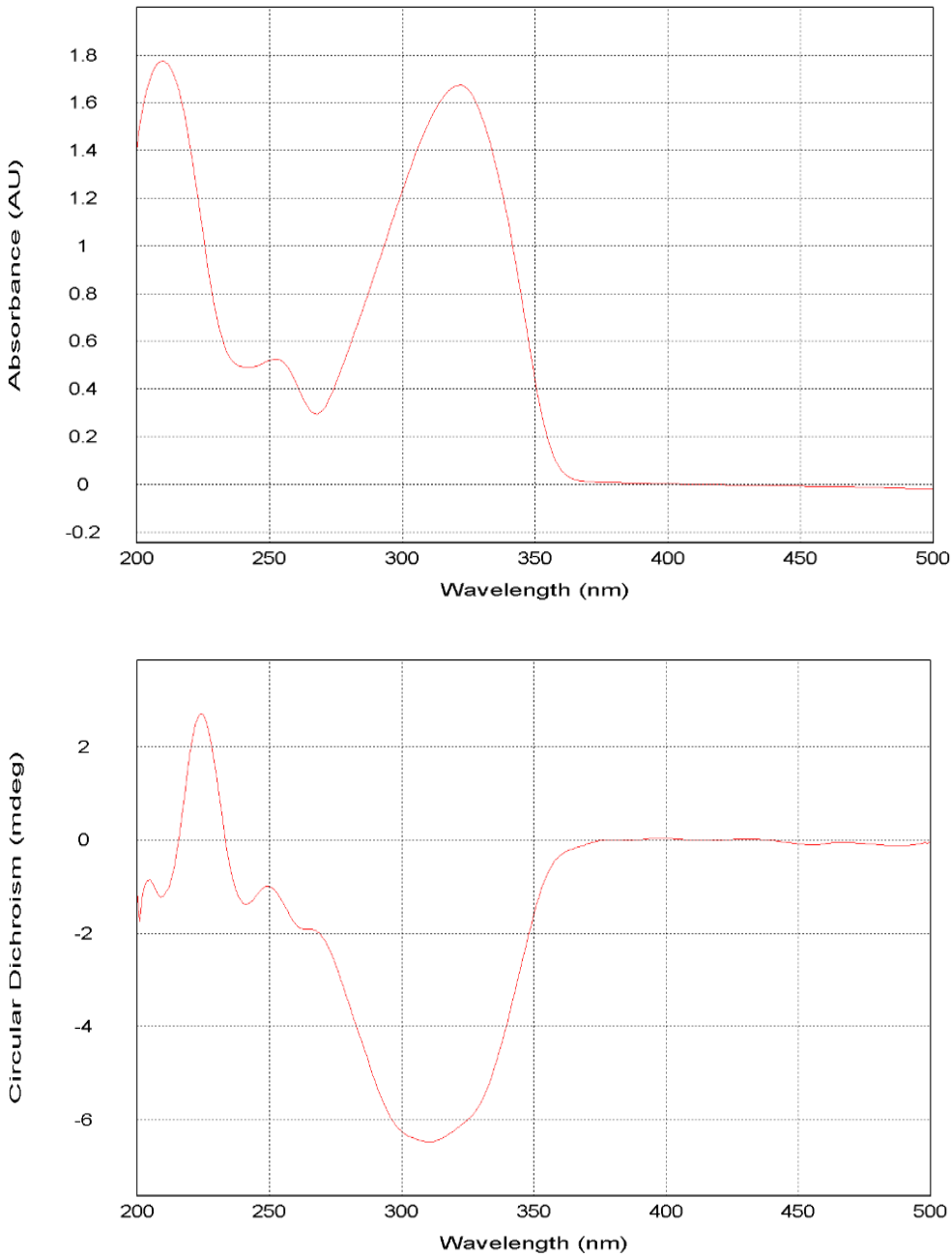

Figure 10. UV and CD spectrum of compound **2**

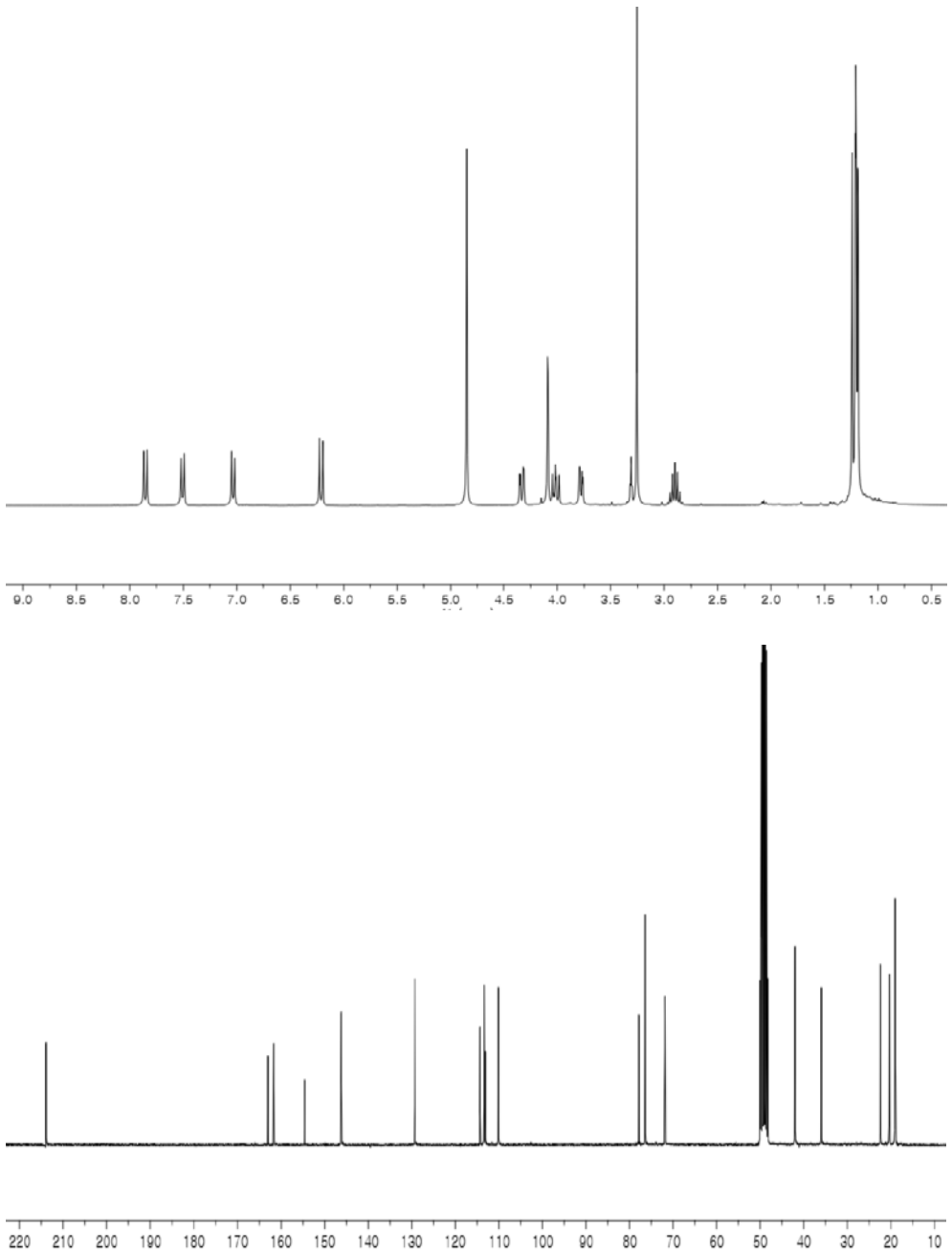

Figure 11. ^{1}H and ^{13}C NMR spectrum of compound **3**

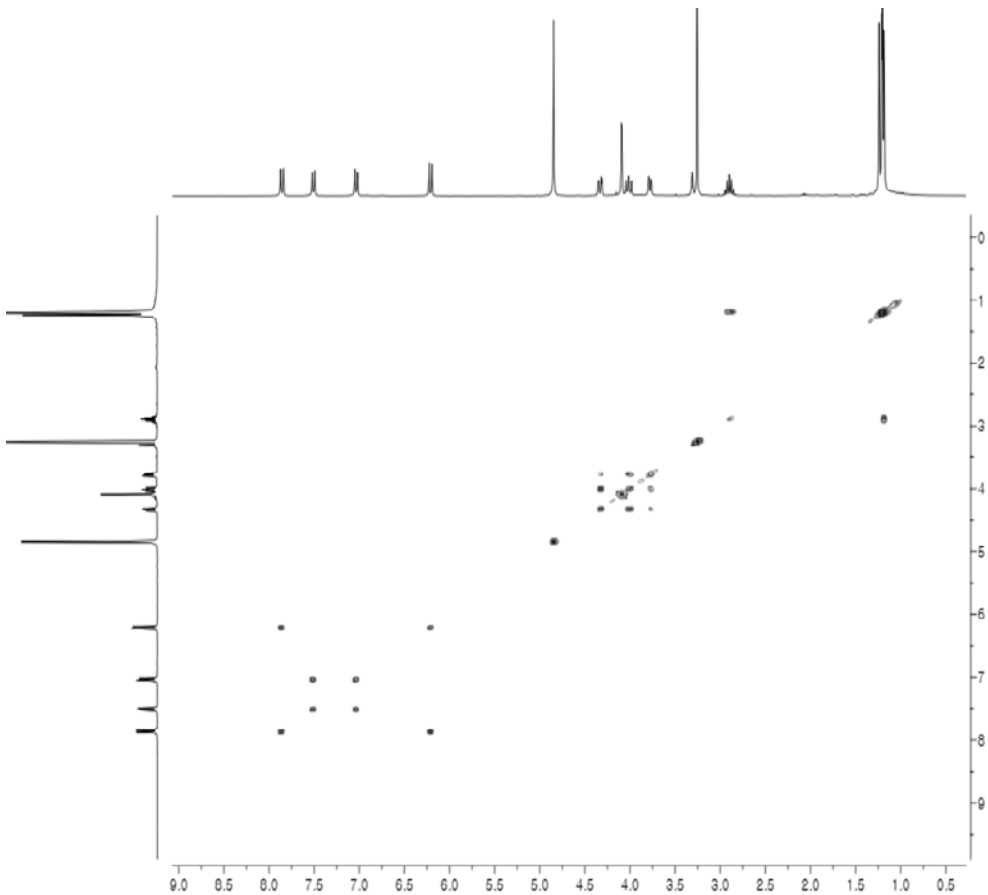

Figure 12. COSY spectrum of compound **3**

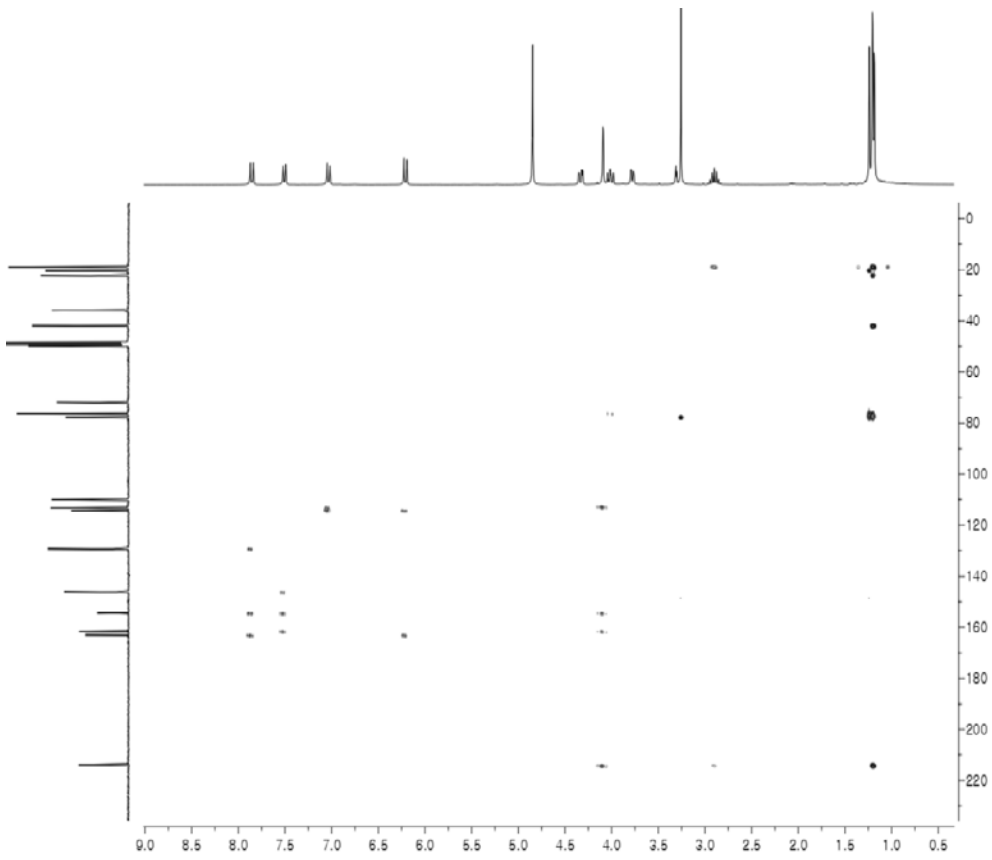

Figure 13. HMBC spectrum of compound **3**

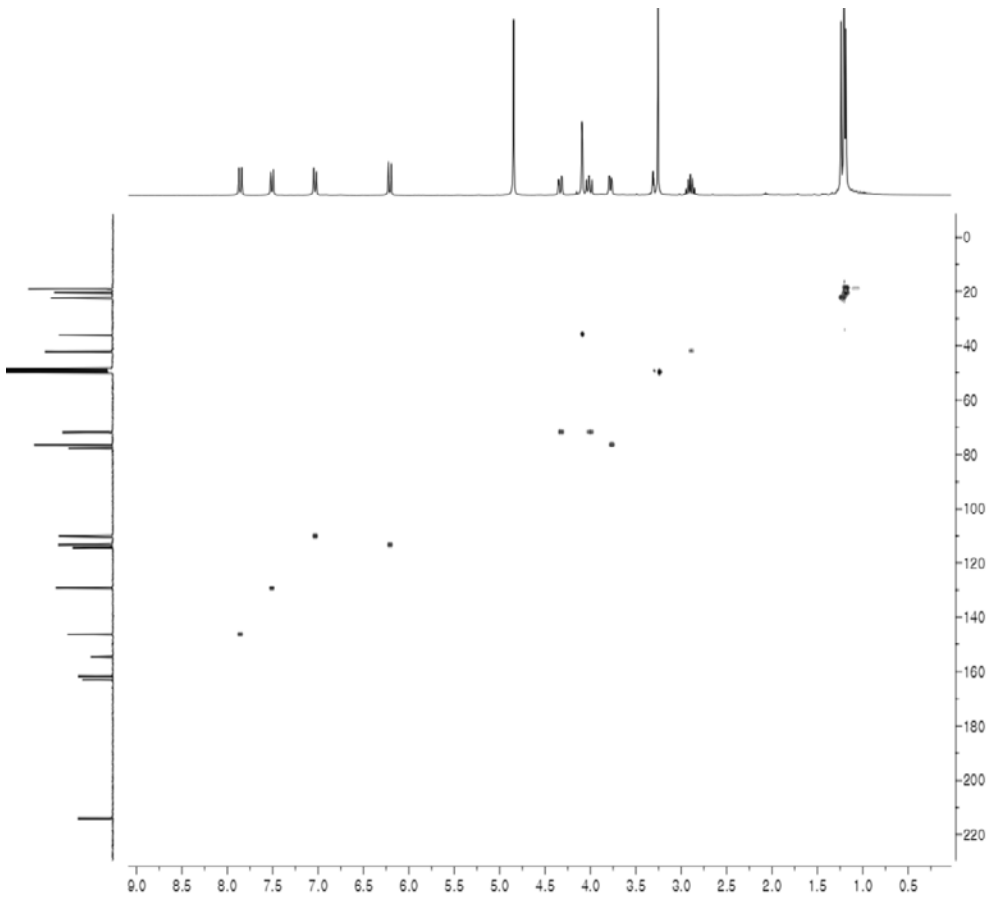

Figure 14. HSQC spectrum of compound **3**

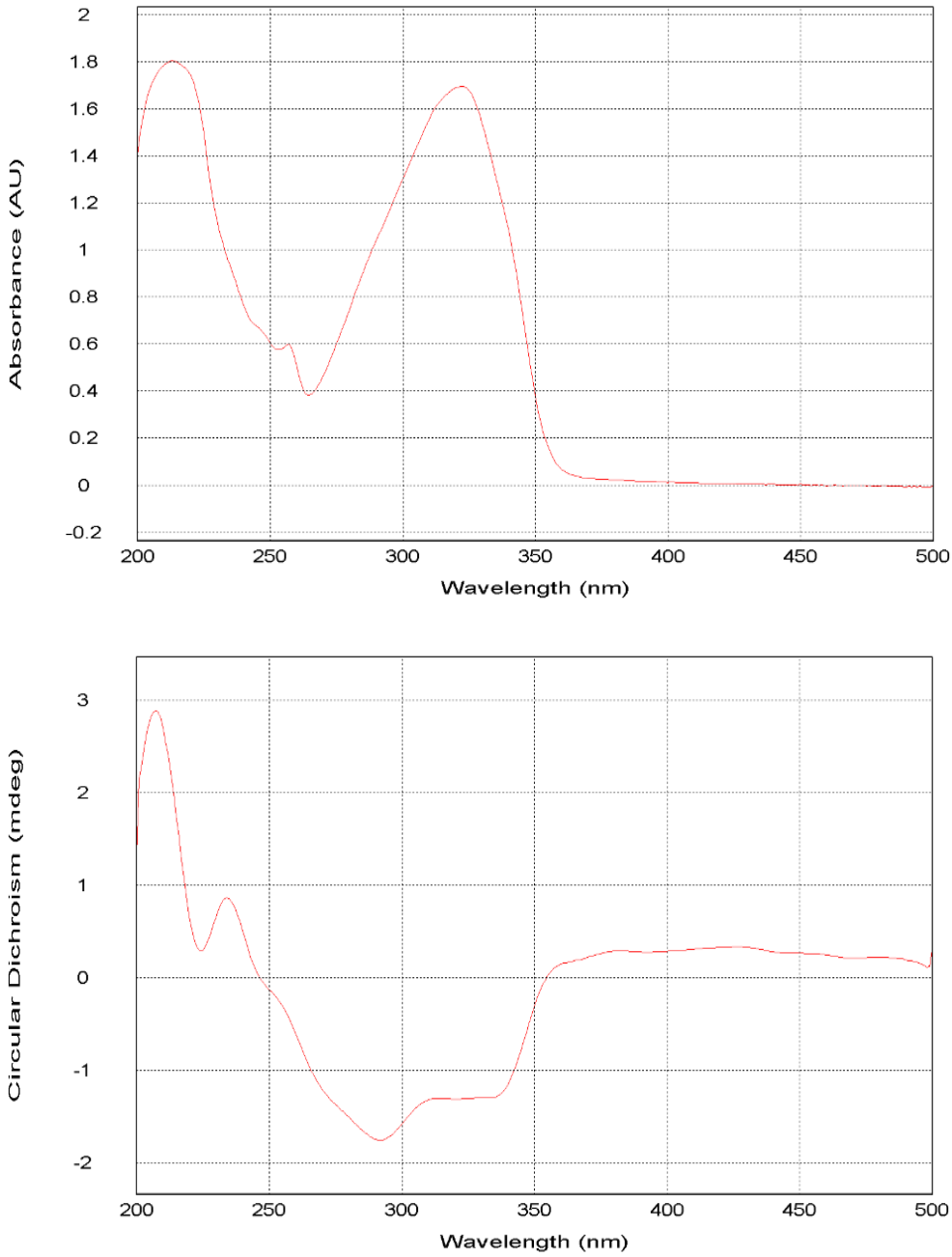

Figure 15. UV and CD spectrum of compound **3**

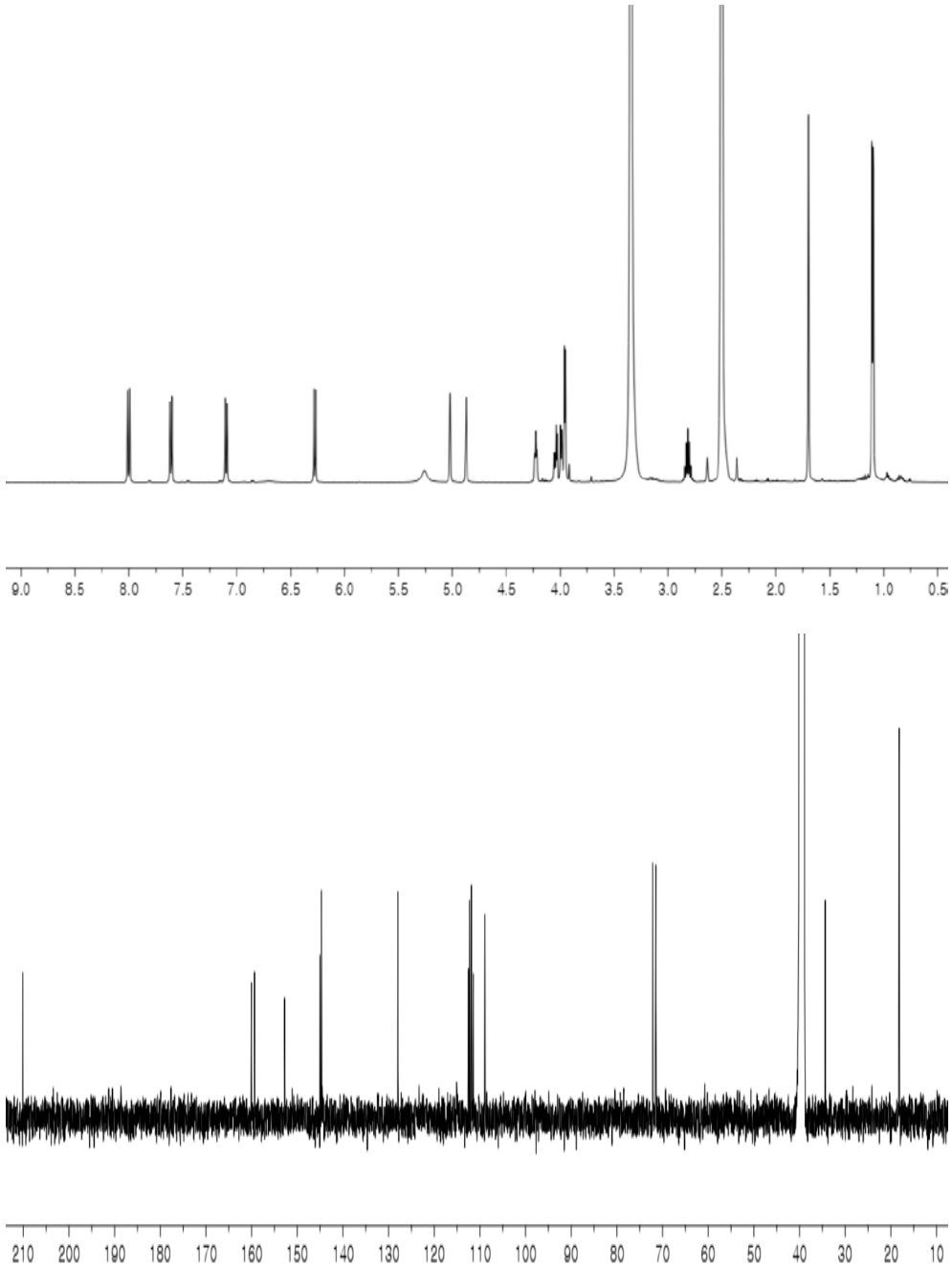

Figure 16. ^{1}H and ^{13}C NMR spectrum of compound **4**

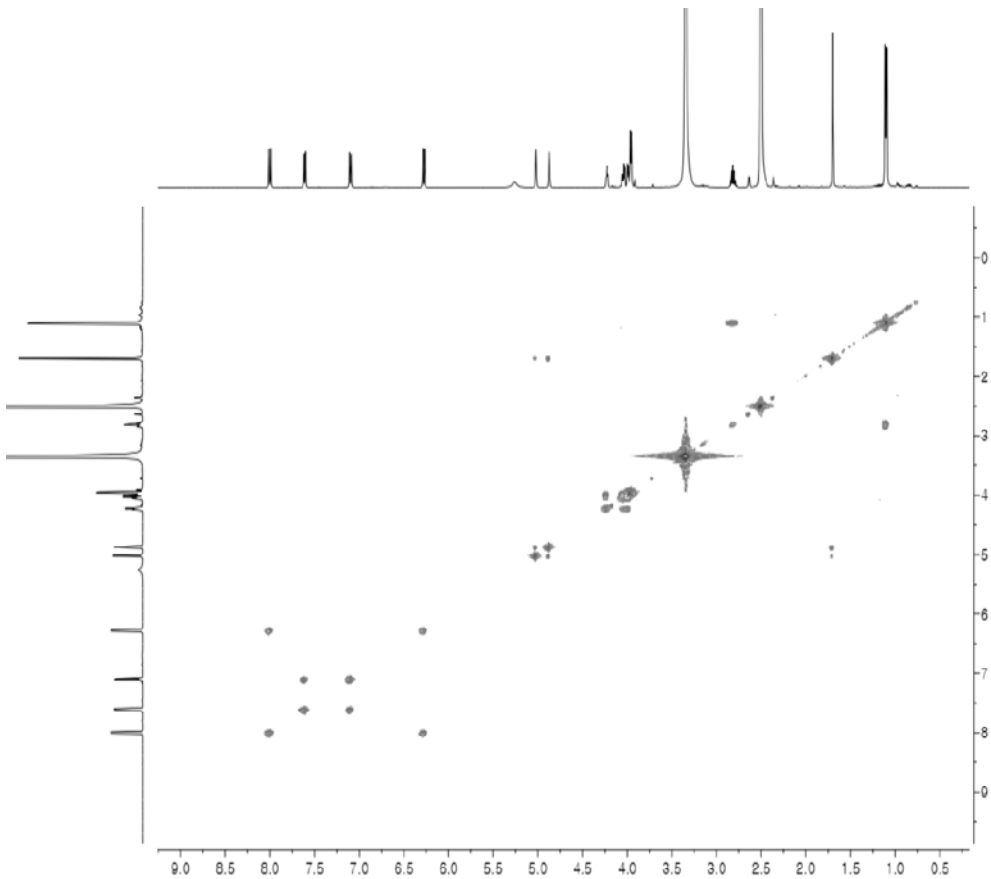

Figure 17. COSY spectrum of compound **4**

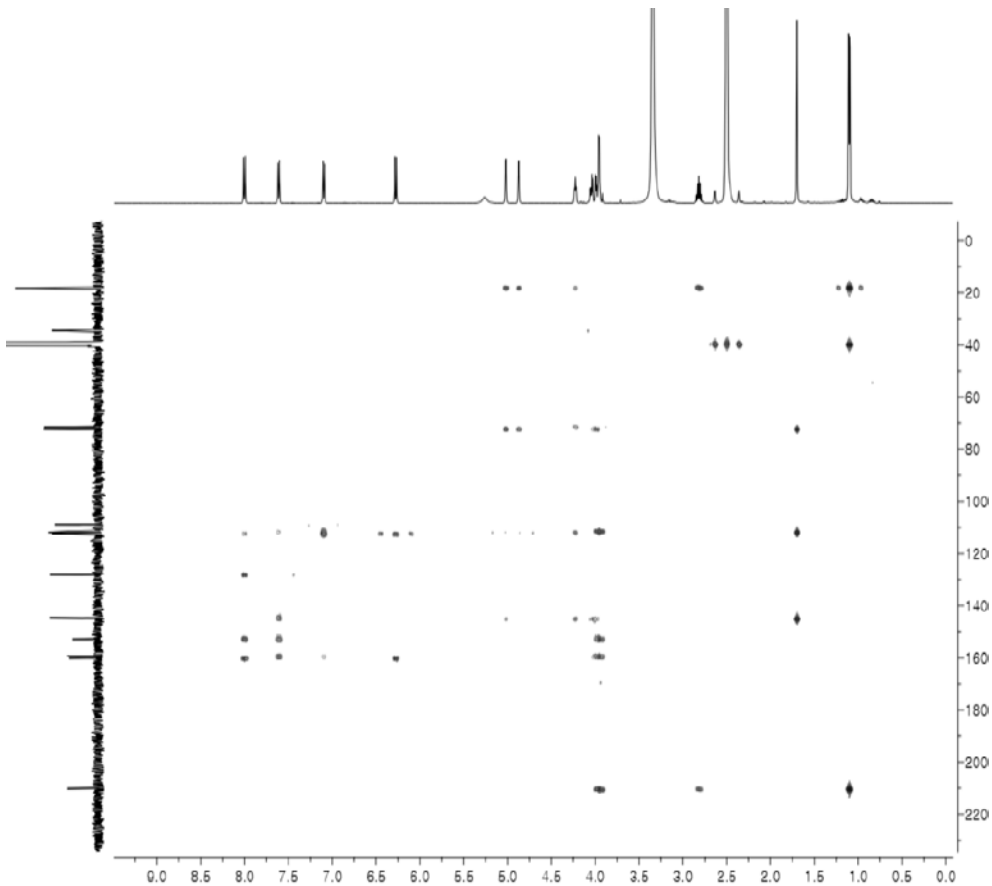

Figure 18. HMBC spectrum of compound **4**

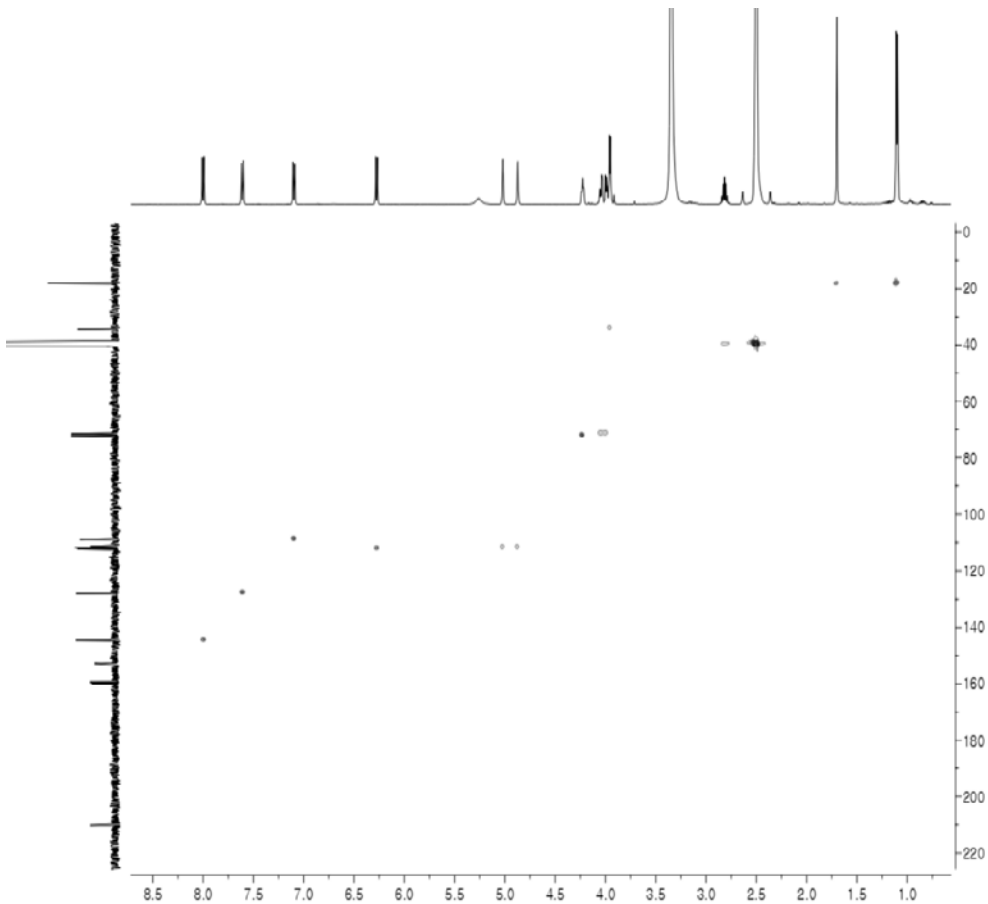

Figure 19. HSQC spectrum of compound **4**

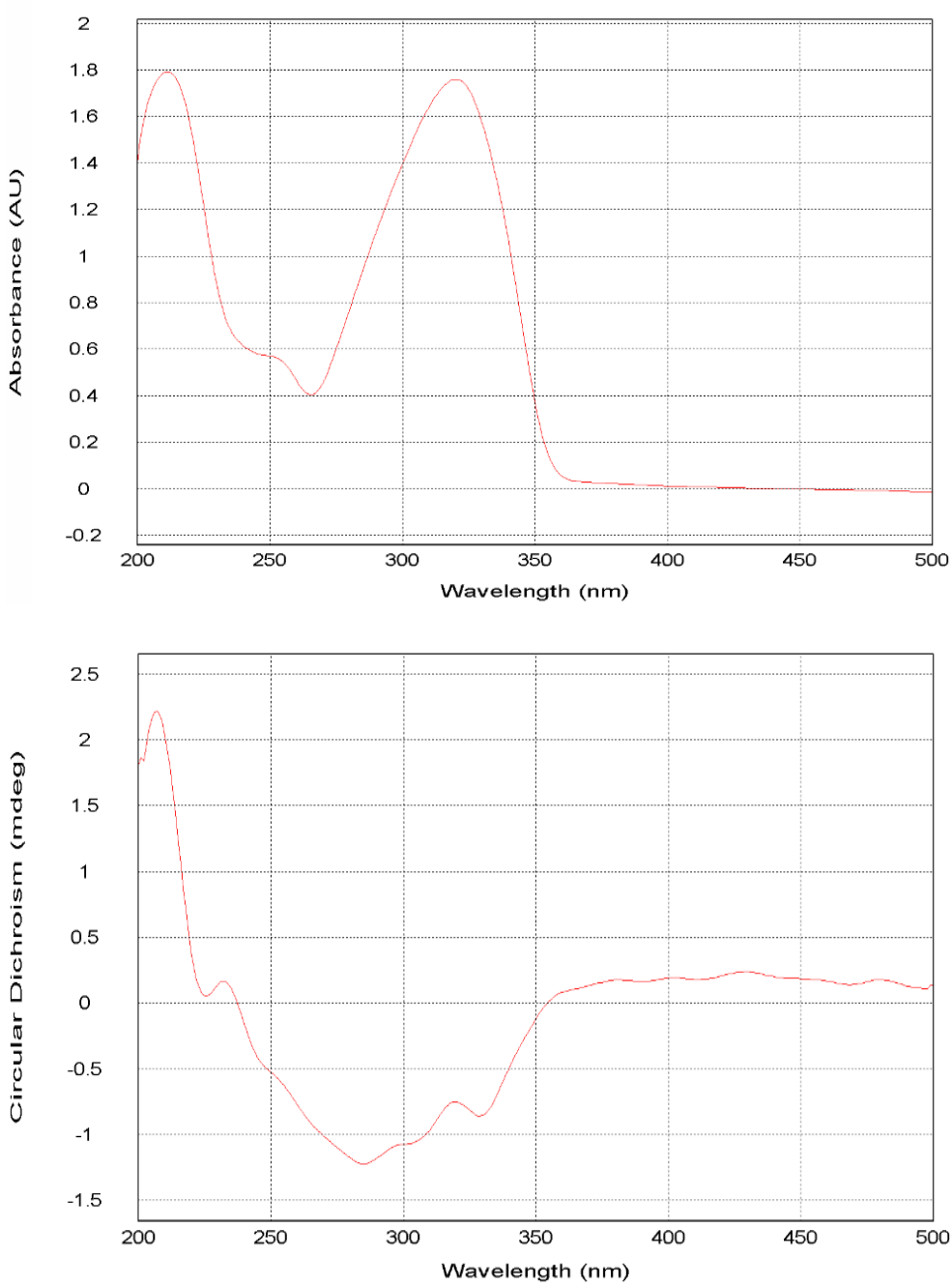

Figure 20. UV and CD spectrum of compound **4**

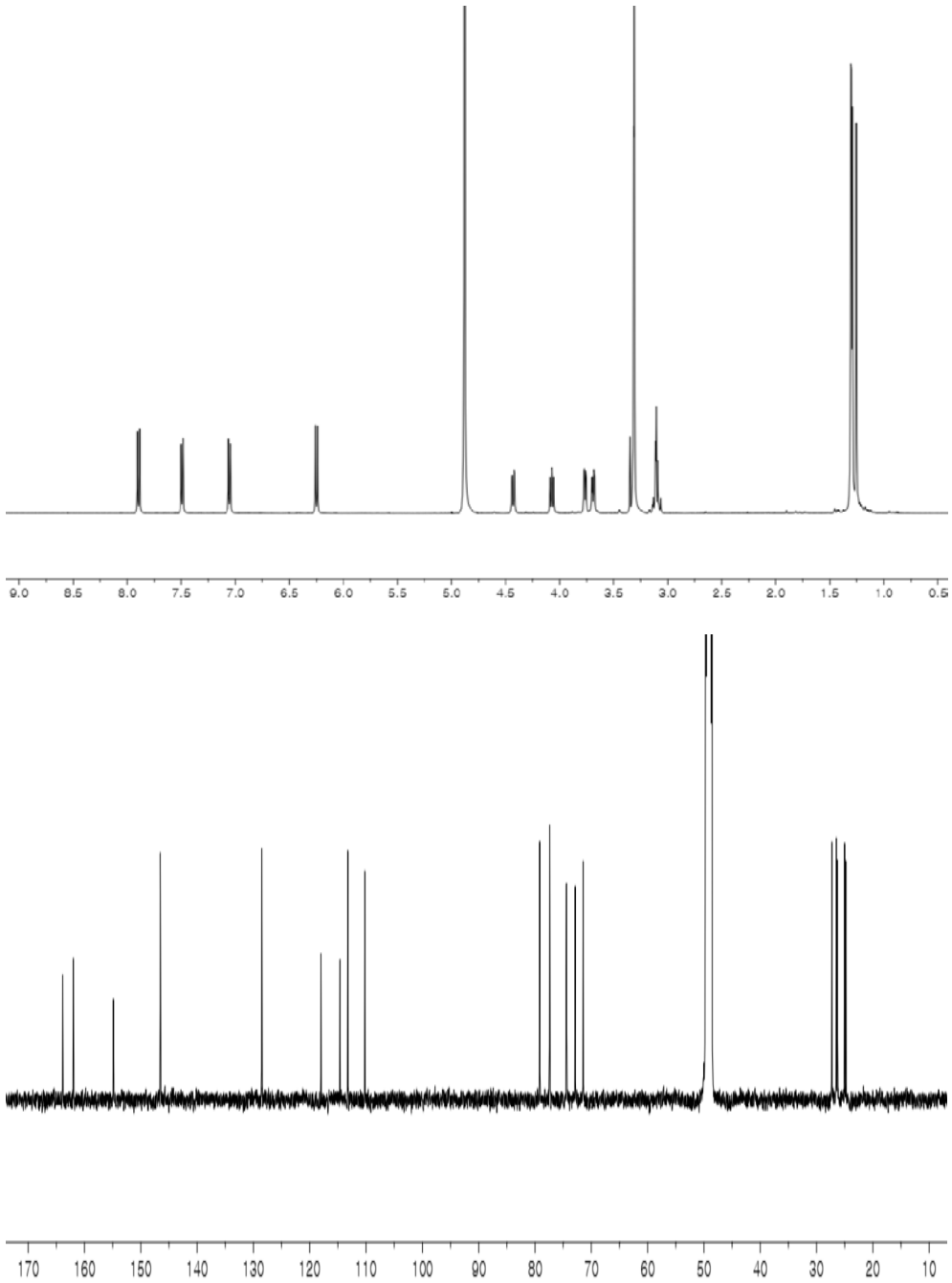

Figure 21. ^{1}H and ^{13}C NMR spectrum of compound **5**

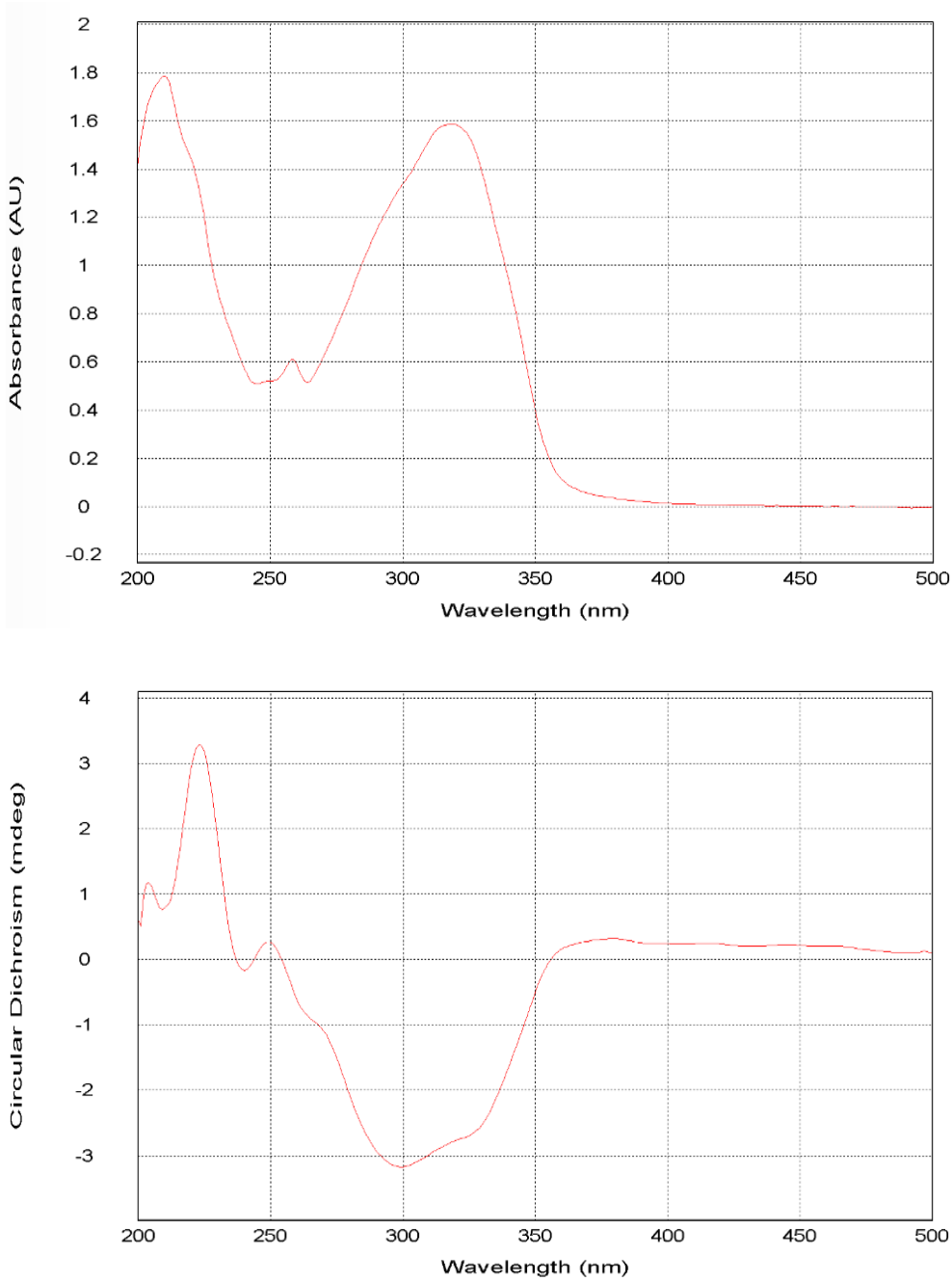

Figure 22. UV and CD spectrum of compound **5**

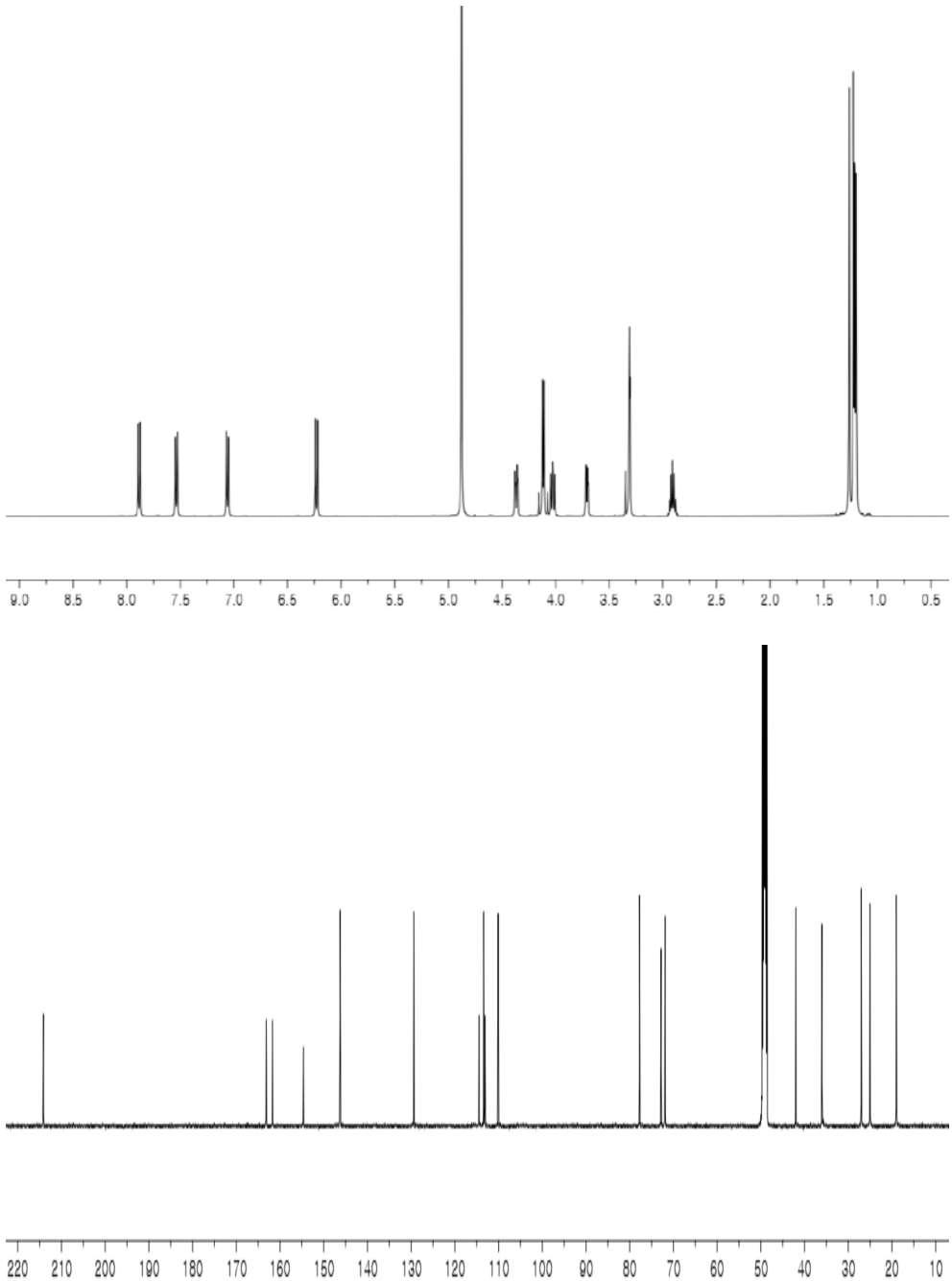

Figure 23. ^{1}H and ^{13}C NMR spectrum of compound **6**

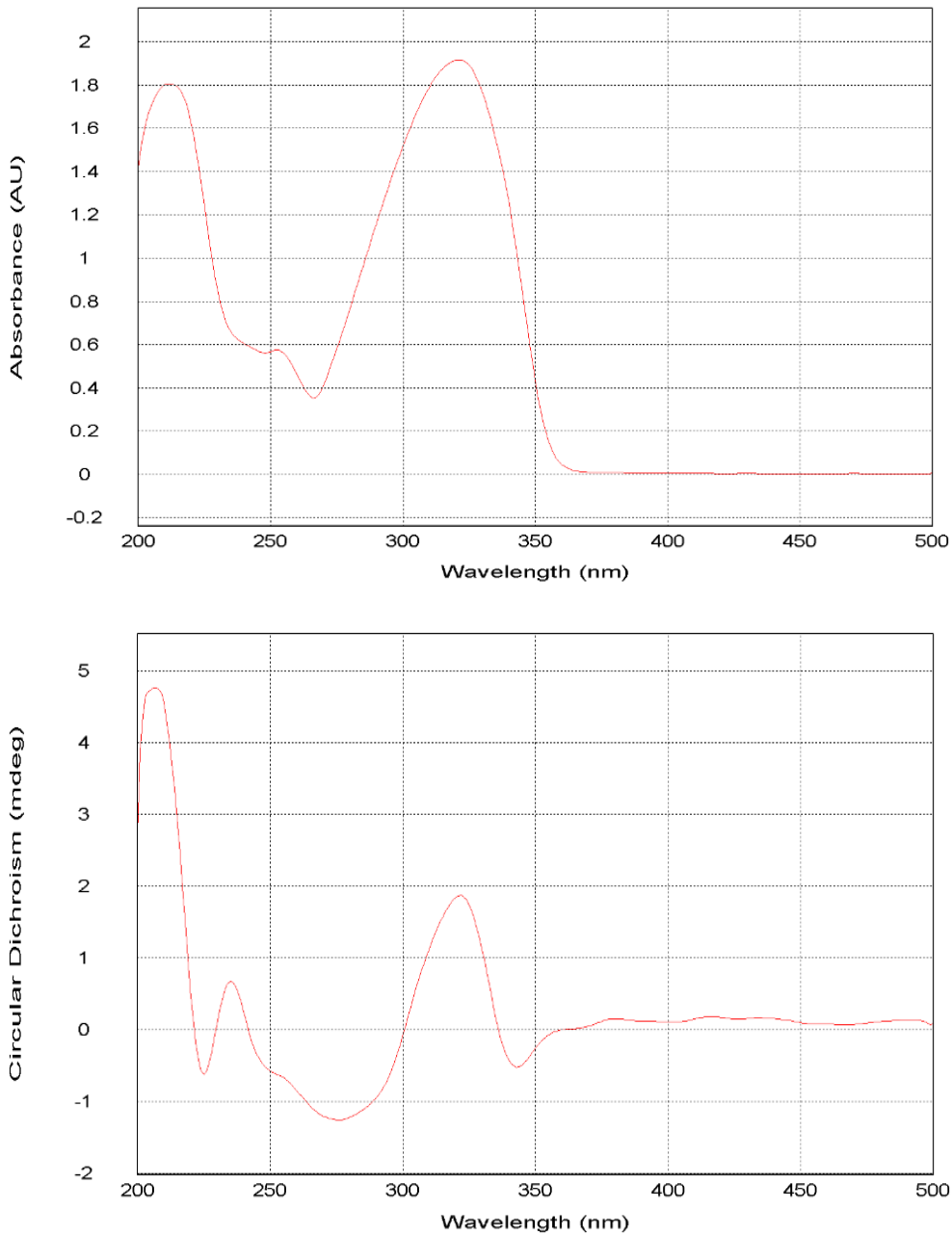

Figure 24. UV and CD spectrum of compound **6**

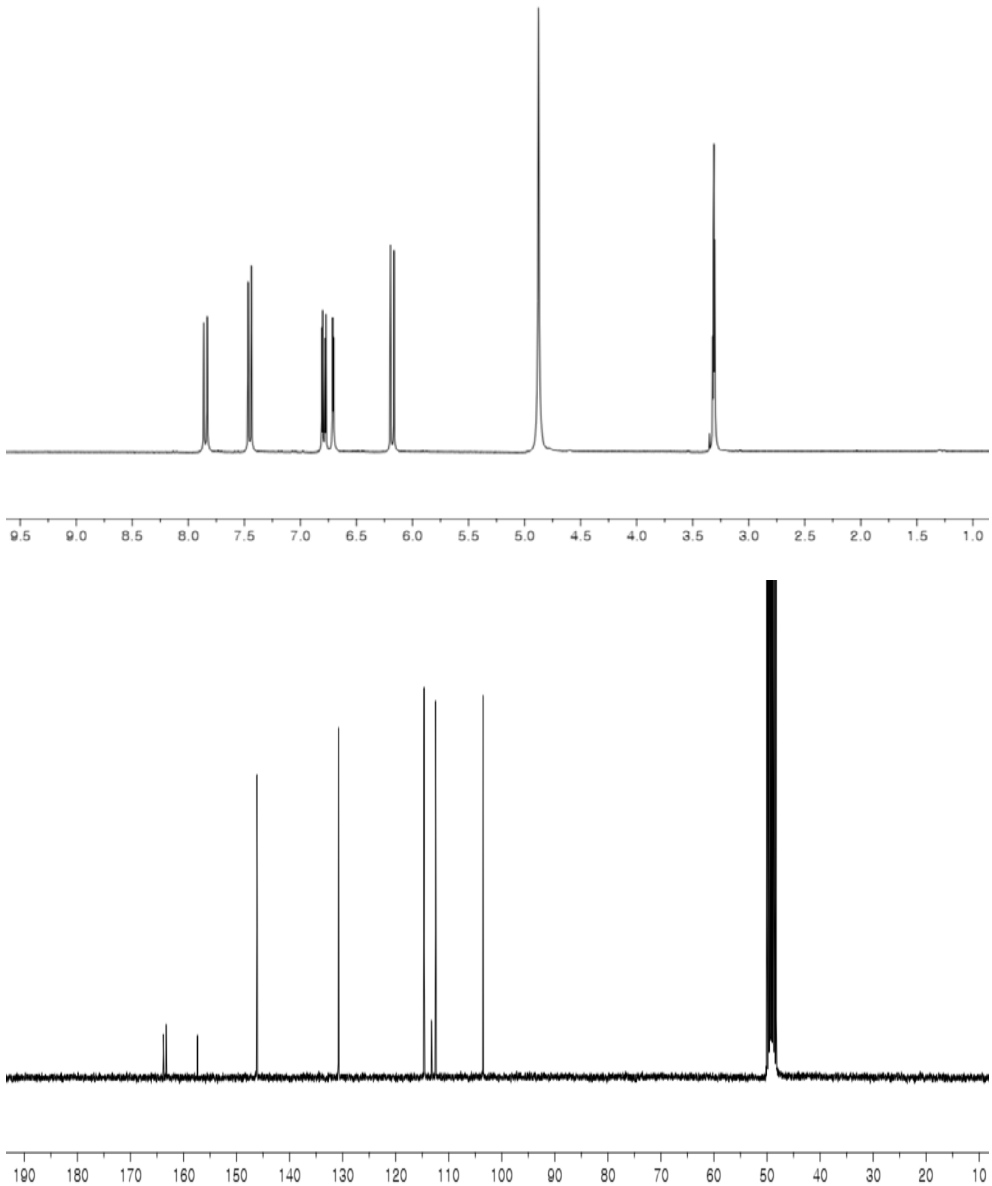

Figure 25. ^{1}H and ^{13}C NMR spectrum of compound **7**

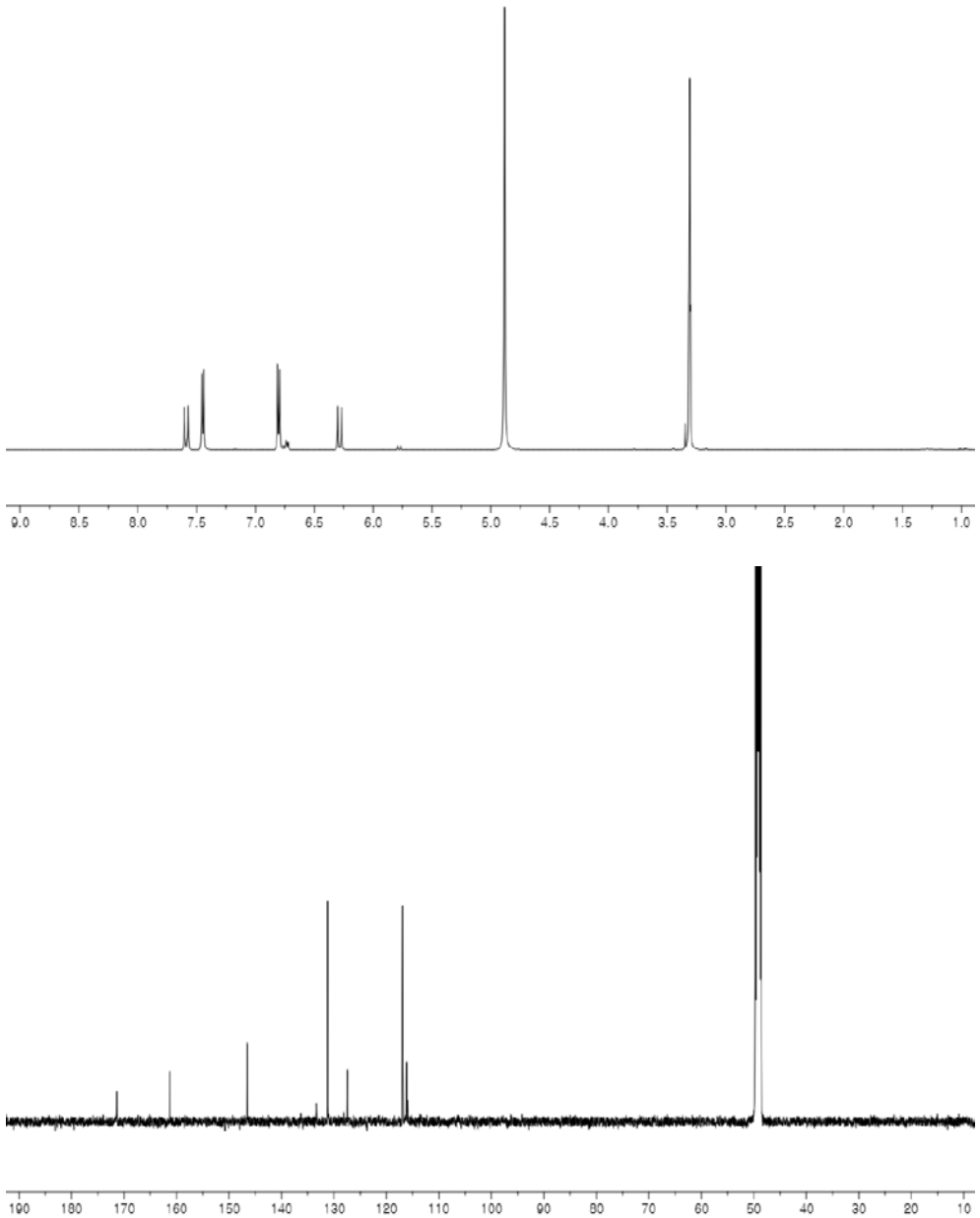

Figure 26. ^{1}H and ^{13}C NMR spectrum of compound **8**

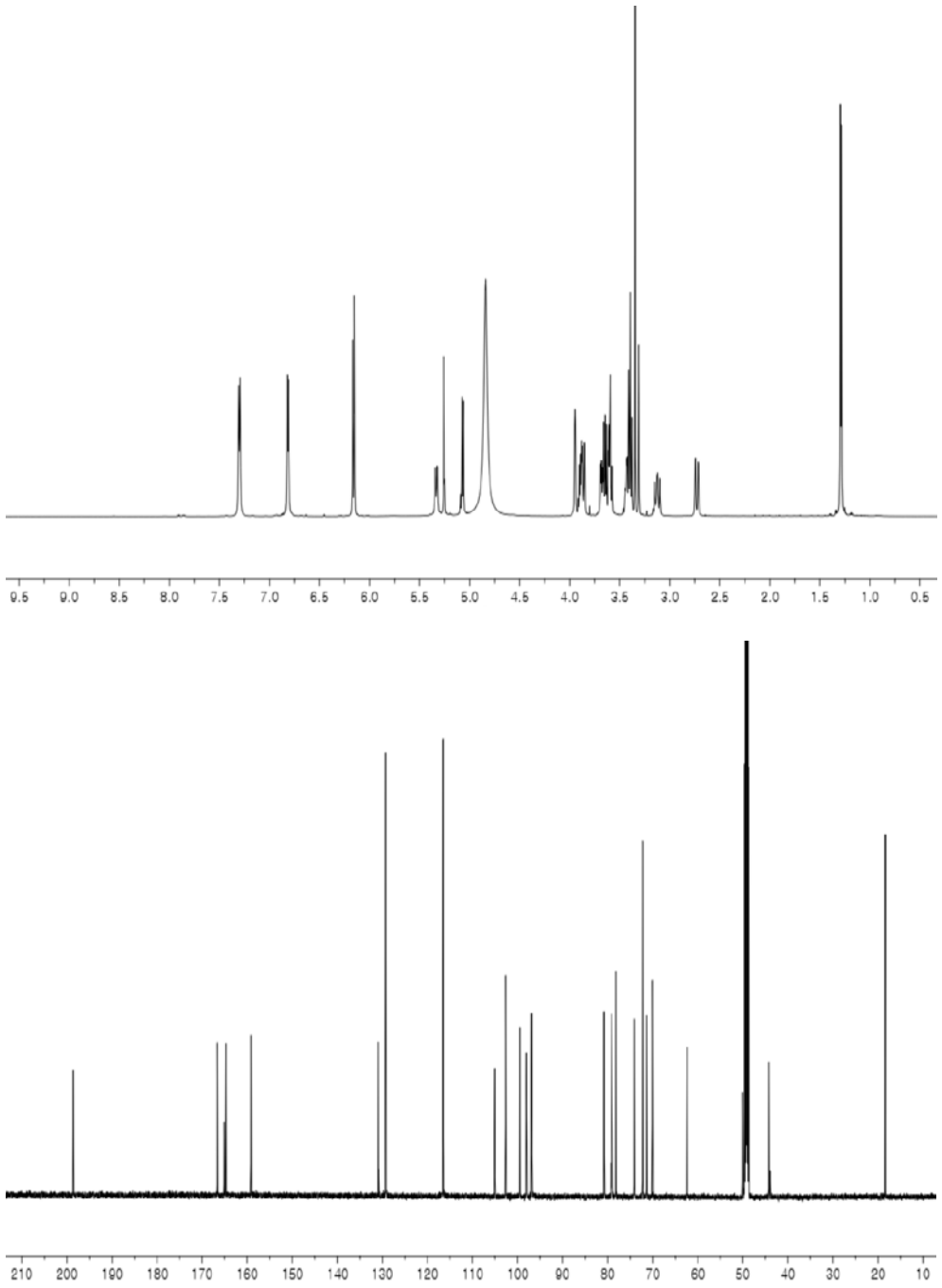

Figure 27. ^{1}H and ^{13}C NMR spectrum of compound **9**

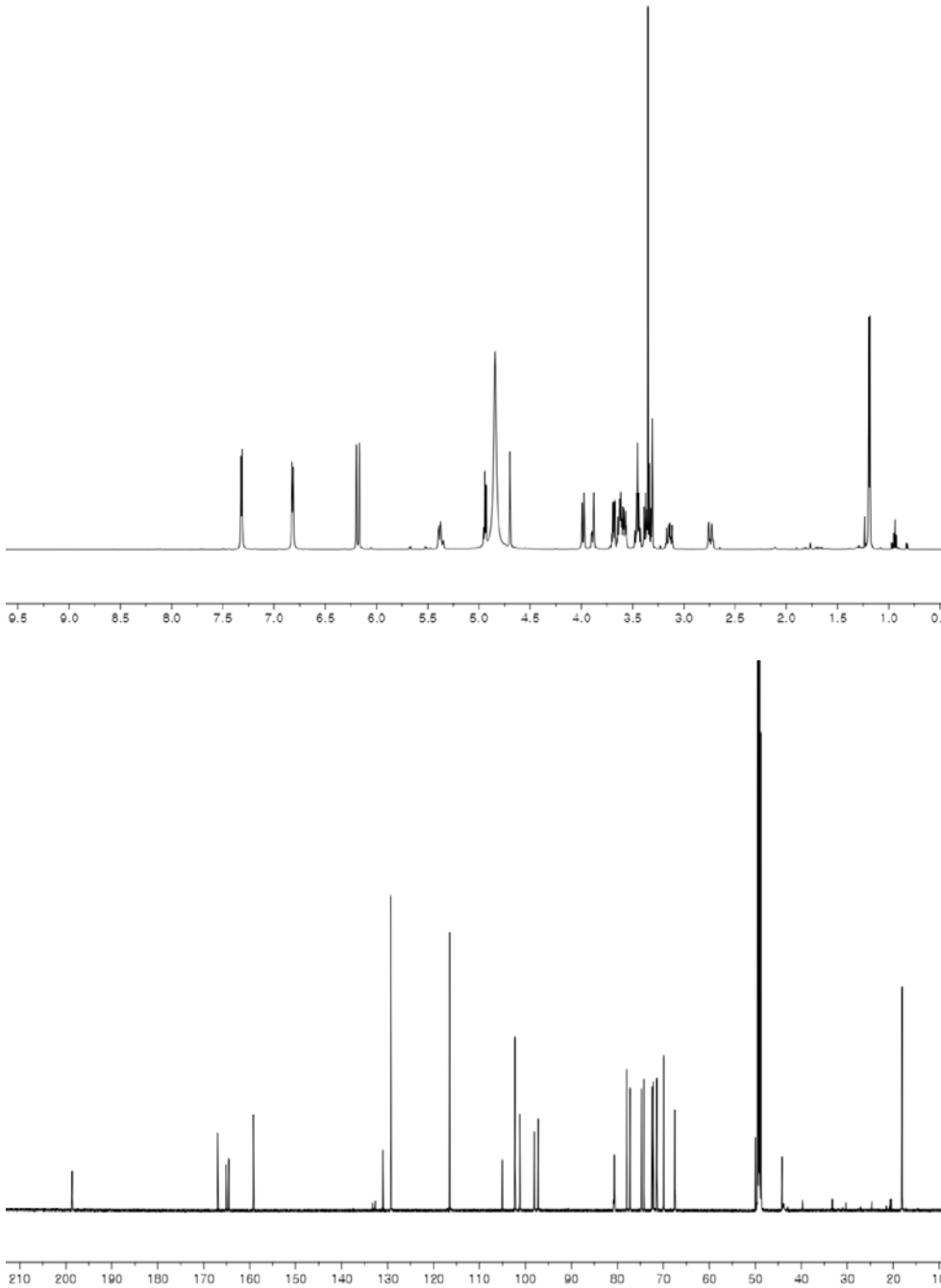

Figure 28. ^{1}H and ^{13}C NMR spectrum of compound **10**

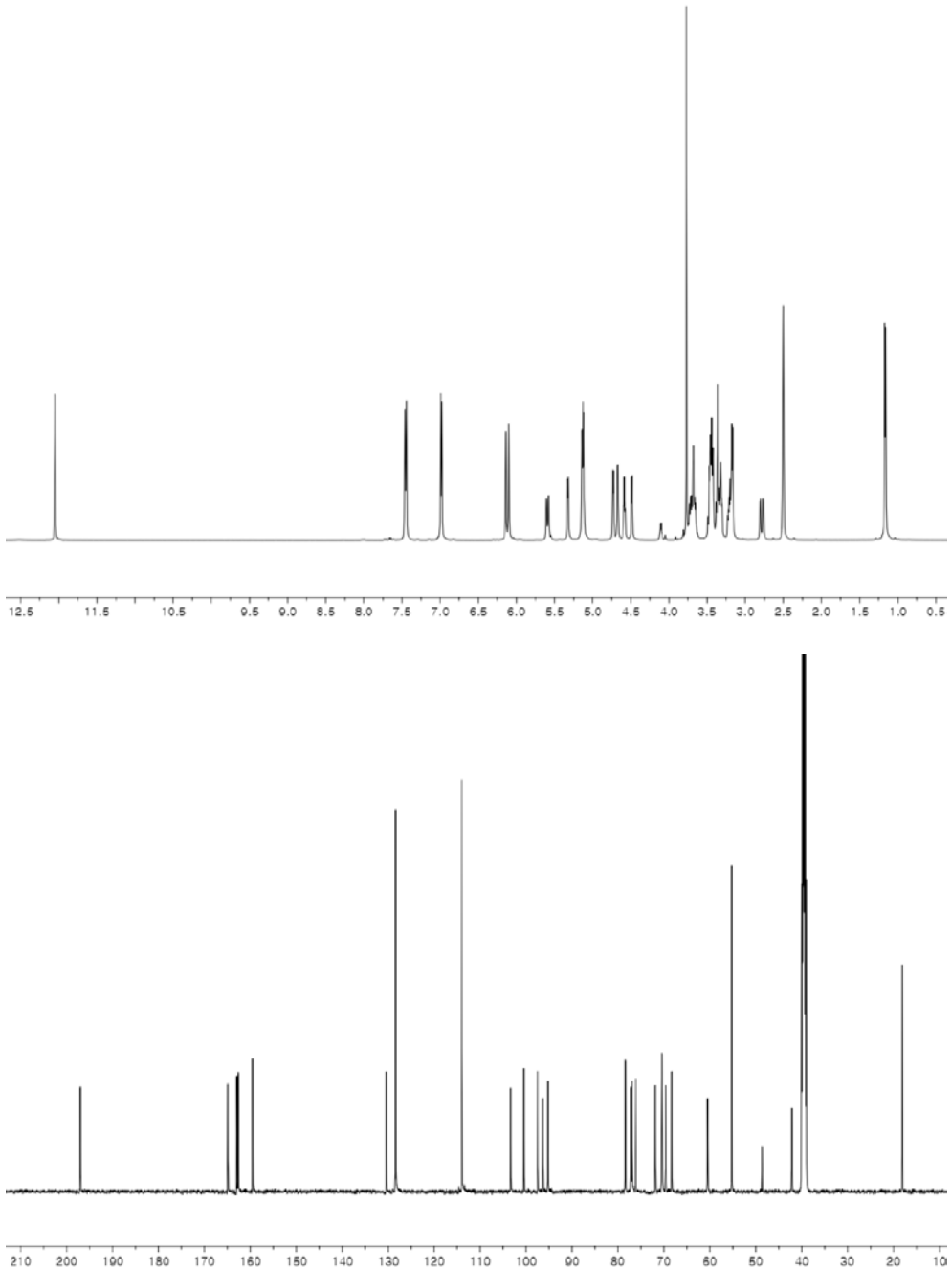

Figure 29. ^{1}H and ^{13}C NMR spectrum of compound **11**

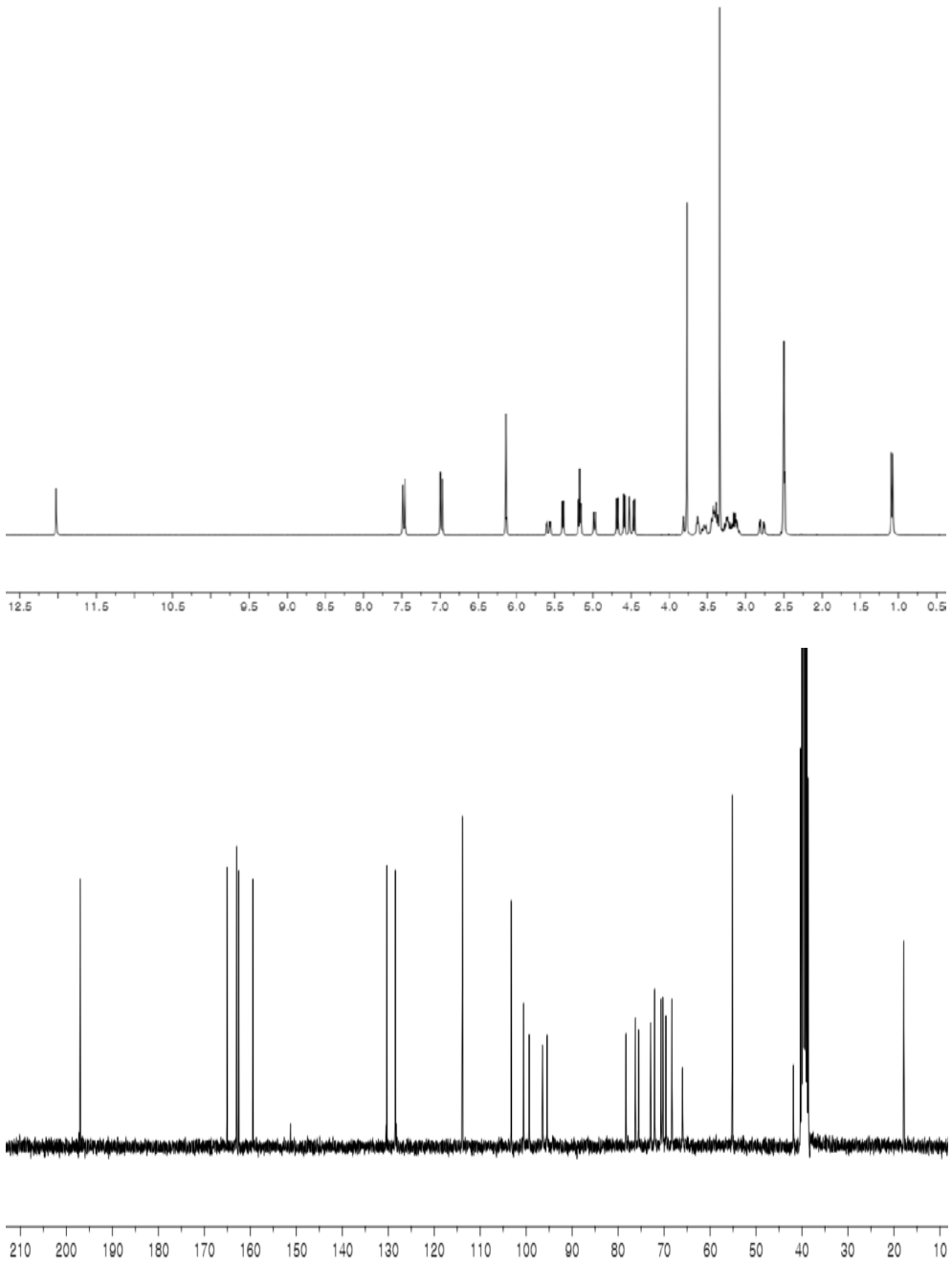

Figure 30. ^{1}H and ^{13}C NMR spectrum of compound **12**

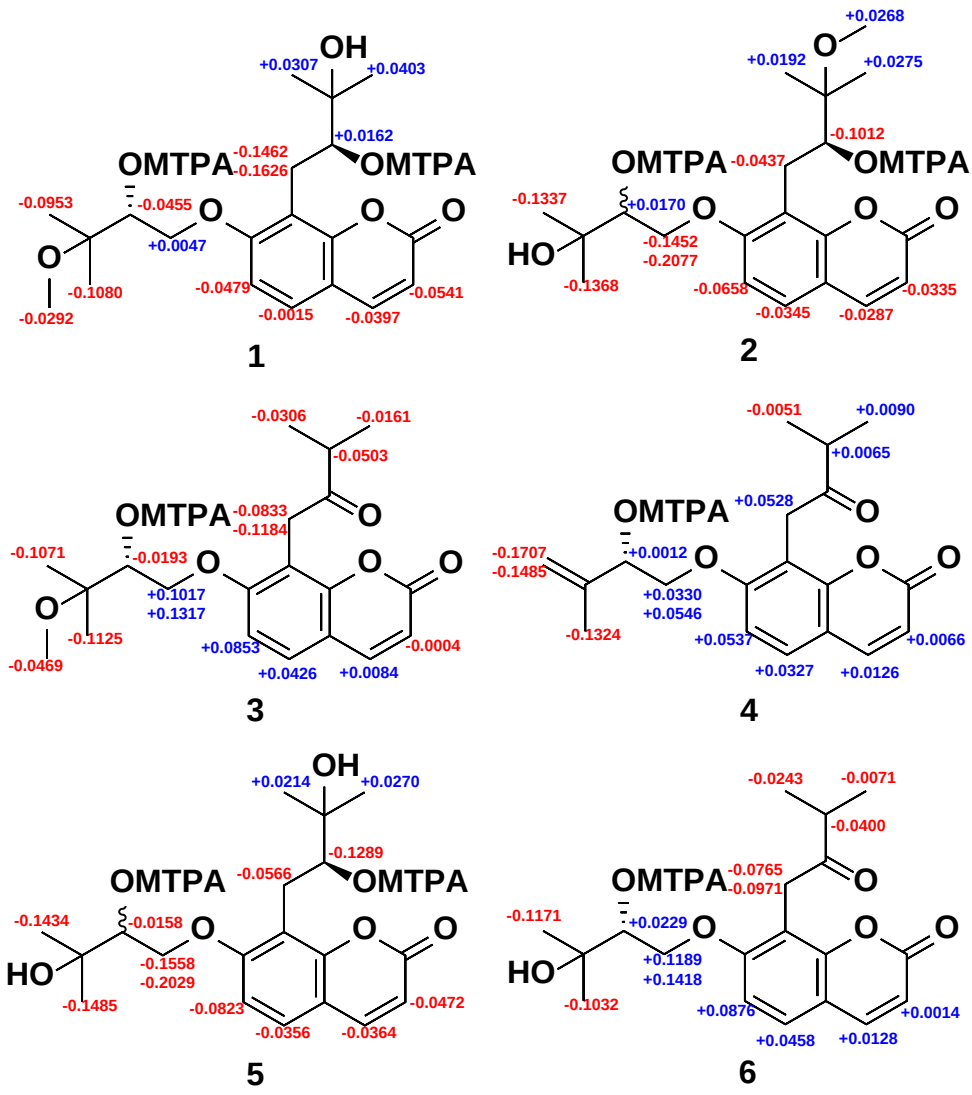

Figure 31. $\Delta\delta$ values (= $\delta_S - \delta_R$; in ppm) obtained for (*S*)- and (*R*)-MTPA esters of compounds **1**-**6**

국문초록

생약 지실의 성분연구

서울대학교 대학원
약학과
천연물과학 전공
이 원 희

Sortase A (SrtA)는 그람양성 세균에 병독성을 갖게 하는 효소로 세균감염을 억제하는 데 있어 중요한 표적효소이다.

전통적으로 다양한 병적 증세의 치료에 사용되는 지실(*Poncirus trifoliate* Rafinesque)은 운향과 (Rutaceae)에 속하는 탱자나무(Trifoliate orange)의 덜 익은 열매로 높은 Srt A 억제를 보였다.

이에 본 연구에서는 Srt A를 억제하는 물질을 지실에서 찾기 위해서 다양한 크로마토그래피 기법을 통해 12종의 물질을 분리하였고, 각각의 분광 자료에 의하여 8종의 coumarins와 4종의 flavanone glycosides 임을 확인하였다. 특히 4종의 coumarins는 자연계에 처음 분리, 보고되는 물질임이 밝혀졌다.

또한 2-methoxy-2-(trifluoromethyl)phenylacetic acid

(MTPA) method를 이용하여 4종의 신규 coumarins와 구조 동정 된 2종의 coumarins의 입체구조를 결정할 수 있었다.

주요어 : herbal medicine, *Poncirus trifoliata* Rafinesque, coumarins, flavanone glycosides, sortase A inhibition.

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